

2016 San Antonio Breast Cancer Symposium

Publication Number: S1-01

Title: Whole exome and transcriptome sequencing of resistant ER+ metastatic breast cancer

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Body: Background: While great strides have been made in the treatment of estrogen receptor-positive (ER+) metastatic breast cancer (MBC), therapeutic resistance invariably occurs. A better understanding of the underlying resistance mechanisms is critical to enable durable control of this disease.

Methods: We performed whole exome sequencing (WES) and transcriptome sequencing (RNA-seq) on metastatic tumor biopsies from 88 patients with ER+ MBC who had developed resistance to one or more ER-directed therapies. For 27 of these patients, we sequenced the treatment-naïve primary tumors for comparison to the resistant specimens. Tumors were analyzed for point mutations, insertions/deletions, copy number alterations, translocations, and gene expression. Detailed clinicopathologic data was collected for each patient and linked to the genomic information.

Results: WES of all metastatic samples demonstrated several recurrently altered genes whose incidence differed significantly from primary, treatment-naïve ER+ breast cancers sequenced in the TCGA study (TCGA). These include ESR1 mutations (n=17, 19.3%; 32.86 fold enrichment, q.value<7.5e-12), CCND1 amplification (n=52, 59.1%; 2.3 fold enrichment, q.value<0.0073), and MAP2K4 biallelic inactivation (n=14, 15.9%; 3.04 fold enrichment, q.value<0.054). Comparing to matched primary samples from the same patient, many alterations were found to be acquired in several cases, including for ESR1, ERBB2, PIK3CA, PTEN, RB1, AKT1, and others. Initial analysis of RNA-seq data from metastatic samples (n=59) allowed classification of individual resistance mechanisms into broader resistance modes based on the observed transcriptional state.

Conclusions: We present a genomic landscape of resistant ER+ MBC using WES and RNA-seq. Multiple genes were recurrently altered in these tumors at significantly higher rates than in ER+ primary breast cancer. When compared with matched primary tumors from the same patient, alterations in these and other genes were often found to be acquired after treatment, suggesting a role in resistance to ER-directed therapies and/or metastasis. Potential resistance mechanisms appear to fall into several categories; integrating RNA-seq data may enhance the ability to identify these categories even when genomic alterations are not identified. Multiple clinically relevant genomic and molecular alterations are identified in metastatic biopsies— with implications for choice of next therapy, clinical trial eligibility, and novel drug targets.

2016 San Antonio Breast Cancer Symposium

Publication Number: S1-02

Title: PrECOG 0102: A randomized, double-blind, phase II trial of fulvestrant plus everolimus or placebo in post-menopausal women with hormone receptor (HR)-positive, HER2-negative metastatic breast cancer (MBC) resistant to aromatase inhibitor (AI) therapy

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Body: Background: Although AIs are an effective treatment for HR-positive MBC, whether used alone or in combination with CDK4/6 inhibitors, resistance to therapy and disease progression invariably develops. Therapeutic options for AI-resistant disease include the m-TOR inhibitor everolimus in combination with the steroidal AI exemestane, or the selective estrogen receptor downregulator (SERD) fulvestrant alone. We hypothesized that the combination of fulvestrant and everolimus would be more effective than fulvestrant alone in AI-resistant MBC.

Methods: Major eligibility criteria included post-menopausal women with HR-positive, HER2-negative MBC, recurrence or progression while receiving prior non-steroidal or steroidal AI therapy, ECOG PS 0-1, and ≤ 1 prior chemotherapy regimen for metastases. All patients received fulvestrant (500mg IM q2 weeks for 3 doses, then q4 weeks) plus oral everolimus (10mg) or placebo (1:1 randomization). Prophylactic corticosteroid mouthwash was not used. Tumor assessment was performed at baseline and every 12 weeks. Treatment continued until progressive disease (PD) by RECIST 1.1 criteria. Patients who discontinued everolimus/placebo due to toxicity continued fulvestrant until PD. The primary endpoint was progression-free survival (PFS), defined as time from start of treatment until progression or death. With accrual of 130 patients (120 eligible), the trial had 90% power to detect an improvement in median PFS from 5.4 months to 9.2 months (1-sided stratified log-rank test, type I error rate 10%), with analysis planned after 98 PFS events.

Results: Of 130 patients treated (64 everolimus, 66 placebo), median age was 61 years (range 35-92), and treatment arms were balanced for stratification factors used in randomization, including ECOG PS 0 vs. 1 (59%/41%), measurable disease (66%), and prior chemotherapy for metastasis (17%). Grade 3/4 AEs were more common in the everolimus arm (53%/3% vs. 23%/3%), including hyperglycemia (16%/0% vs. 0%), stomatitis (11%/0% vs. 0%), hypertriglyceridemia (9%/2% vs. 0%), lymphopenia (9%/0% vs. 0%), and pneumonitis (6%/2% vs. 0%). There were 3 grade 5 events (2 everolimus, 1 placebo arm), none of which were attributed to therapy. Selected grade 2 events of interest included fatigue (17% vs. 6%), hyperglycemia (6% vs. 0%), and stomatitis (6% vs. 0%). Everolimus/placebo was discontinued for adverse events, patient withdrawal, or physician discretion in 39% in the everolimus arm and 21% in the placebo arm. After 98 PFS events, median PFS was 10.4 months in the everolimus arm versus 5.1 months in the placebo arm (hazard ratio: 0.61, 95% C.I. 0.40 – 0.92; stratified logrank $p = 0.02$).

Conclusions: The addition of everolimus to fulvestrant significantly improved PFS in post-menopausal women with AI-resistant MBC. Everolimus used without prophylactic corticosteroid mouthwash exhibited a similar rate of oral mucositis and overall AE profile when combined with fulvestrant as when combined with exemestane.

Keywords: advanced breast cancer, PrECOG 0102, endocrine resistance, everolimus, exemestane, hormone receptor-positive, mTOR inhibitor, postmenopausal.

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Publication Number: S1-03

Title: First results from the multicenter phase III DATA study comparing 3 versus 6 years of anastrozole after 2-3 years of tamoxifen in postmenopausal women with hormone receptor-positive early breast cancer

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Body: Background. Even in view of the recent findings of the MA.17R trial, the impact of prolonged aromatase inhibitor (AI) therapy after prior tamoxifen in hormone receptor-positive early breast cancer remains insufficiently clear.

Methods. In this open-label phase III study, we randomly assigned 1912 postmenopausal women with hormone receptor-positive breast cancer after 2-3 years of adjuvant tamoxifen to either 3 or 6 years of anastrozole therapy. The primary endpoint was the adapted disease-free survival (ADFS). This was defined as the DFS beyond 3 years after randomization to AI therapy because initially all patients received the same AI therapy for 3 years. ADFS events included (non-) invasive breast cancer recurrences (local, regional, distant), second primary (non-) invasive (breast) cancers, and death of any cause. The study was designed to detect an increase of the ADFS in the 6-year versus the 3-year anastrozole group corresponding with a hazard ratio (HR) of 0.60. The HRs and the corresponding 95% confidence intervals (CIs) were estimated with stratified Cox proportional-hazard models according to intention-to-treat.

Results. Patients were randomized from July 2006 till August 2009. Three years after randomization 1663 patients had no DFS events, with an equal distribution between the treatment arms. The patient and tumor characteristics were well balanced. The median age at randomization was 57 years ($P_5 = 45$ years, $P_{95} = 76$ years), the median primary tumor size was 21 mm ($P_5 = 10$ mm, $P_{95} = 50$ mm), 67% of the patients had node-positive disease, and in 2% the tumor was HER2-positive (14% unknown); 64% of the patients had received adjuvant chemotherapy and <1% adjuvant trastuzumab. The median adapted follow-up was 4.1 years ($P_5 = 2.9$ years, $P_{95} = 5.8$ years). No unexpected safety issues were seen. The 5-year ADFS was 79% in the 3-year and 83% in the 6-year anastrozole treatment group, yielding a HR for ADFS-event of 0.78 (95% CI 0.61 to 1.00; $p = 0.0528$). In patients with node-positive disease ($N = 1117$), the HR for ADFS-event was 0.71 (95% CI 0.53 to 0.96; $p=0.0232$), in N0 disease ($N=546$) 1.01 (95% CI 0.62 to 1.63; $p=0.9817$) and in patients with both ER and PR positive breast cancer ($N = 1264$) 0.68 (95% CI 0.51 to 0.90; $p=0.0072$). The 5-year adapted overall survival was not different between the treatment groups.

Conclusion. These findings do not yet support the use of extended adjuvant AI prescription after 5 years of sequential endocrine therapy for postmenopausal patients with hormone receptor-positive breast cancer, but suggest benefit for a selected group of patients. Continued follow-up is needed to assess long-term efficacy and safety.

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Publication Number: S1-04

Title: Optimal duration of extended letrozole treatment after 5 years of adjuvant endocrine therapy; results of the randomized phase III IDEAL trial (BOOG 2006-05)

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Body: Despite the success of adjuvant endocrine therapy in early breast cancer, approximately 50% of all recurrences still occur after the initial 5 years of follow-up. Earlier studies confirmed that endocrine therapy extension after 5 years of adjuvant tamoxifen with either tamoxifen or aromatase inhibitors up to 10 years leads to an improvement in survival. However, aromatase inhibitors are currently standard of care in the initial 5 years of adjuvant therapy, and the benefit of extended use beyond 5 years of AI based therapy is still debated. The randomized phase III IDEAL trial was designed to study the optimal duration of extended adjuvant endocrine therapy after the initial 5 years of any endocrine therapy.

Between April 2007 and November 2011, 1824 postmenopausal, HR-positive early breast cancer patients were included by 74 hospitals in the Netherlands. Enrolled patients earlier received 5 years of any endocrine therapy (87.9% AI based), completed this treatment no longer than 2 years before randomization, and did not have any recurrence at the moment of inclusion. The included patients were randomly allocated to either 2.5 or 5 years of extended letrozole (daily 2.5mg). Primary outcome was disease free survival (DFS), secondary endpoints were overall survival (OS), distant disease free survival (DDFS), contralateral breast cancer and safety.

Results & Discussion: Results will become available soon.

2016 San Antonio Breast Cancer Symposium

Publication Number: S1-05

Title: A randomized, double-blinded, placebo-controlled clinical trial of extended adjuvant endocrine therapy (tx) with letrozole (L) in postmenopausal women with hormone-receptor (+) breast cancer (BC) who have completed previous adjuvant tx with an aromatase inhibitor (AI): Results from NRG Oncology/NSABP B-42

Mamounas EP P, Bandos H, Lembersky BC C, Geyer, Jr CE E, Fehrenbacher L, Graham ML L, Chia SL L, Brufsky AM M, Hennessy BT T, Soori GS S, Dakil SR R, Seay TE E, Wade, III JL L, McCarron EC C, Paik S, Swain SM M, Wickerham DL and Wolmark N. NRG Oncology/NSABP (NSABP Legacy Trials Are Now Part of the NRG Oncology Portfolio), Pittsburgh, PA; UF Cancer Center at Orlando Health, Orlando, FL; University of Pittsburgh, Pittsburgh, PA; The University of Pittsburgh Cancer Institute, Pittsburgh, PA; Massey Cancer Center, Virginia Commonwealth University, Richmond, VA; Kaiser Permanente Oncology Clinical Trials Northern California - Vallejo, Vallejo, CA; Southeast Cancer Control Consortium, Goldsboro, NC; British Columbia Cancer Agency (BCCA), Vancouver, BC, Canada; Cancer Trials Ireland (Formerly known as Irish Clinical Oncology Research Group - ICORG), Dublin, Ireland; Missouri Valley Cancer Consortium, Omaha, NE; CCCOP, Wichita Cancer Center of Kansas, Wichita, KS; Georgia NCI Community Oncology Research Program, Atlanta, GA; CCOP, Central Illinois, Decatur, IL; MedStar Franklin Square Medical Center, Weinberg Cancer Institute, Baltimore, MD; Severance Biomedical Science Institute and Yonsei University College of Medicine, Seoul, Korea; Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC and Allegheny Health Network Cancer Institute, Pittsburgh, PA.

Body: Background: Extending adjuvant endocrine therapy (tx) after 5 yrs of tamoxifen (Tam) with either Tam or an AI improves disease-free survival (DFS) in early-stage breast cancer (BC). However, optimal duration of adjuvant AI tx beyond 5 yrs is unknown. NSABP B-42 aimed to determine whether 5 yrs of letrozole (L) v placebo (P) improves DFS in patients (pts) who have completed 5 yrs of hormonal tx (with either AI or TAM→AI).

Methods: Postmenopausal pts with stage I-III, hormone-receptor (+) BC, disease-free after 5 yrs of either AI or Tam for ≤3 years→AI for the remainder of 5 yrs, were randomized to L 2.5 mg or P daily for an additional 5 yrs. Stratification was by pathological nodal status, prior adjuvant Tam or not, and baseline dexamethasone T scores (>-2.0, ≤-2.0 SD). Primary endpoint was DFS including local, regional, distant recurrence (DR), second primary cancers, and deaths from any cause as first event. Secondary endpoints included overall survival (OS), BC-free interval (BCFI including recurrence or contralateral BC as first event), DR, osteoporotic fractures (OF), and arterial thrombotic (AT) events. Differences in DFS, OS, BCFI, DR, OF, and AT between L and P were assessed by the stratified log-rank tests and Cox proportional hazards models. Statistical significance level for DFS was set at 0.0418 as per statistical plan.

Results: From 9/06-1/10, 3966 pts were randomized (34% were <60 yrs, 57% were node-negative, 39% received prior TAM, 14% were HER-2 neu positive). Median follow-up for 3923 pts included in efficacy analyses was 6.9 years. As of 8/16, 631 DFS events occurred (L=292, P=339); L did not result in statistically significant increase in DFS v P (HR=0.85; 95% CI 0.73, 0.999; P=0.048), even after adjusting for age or surgery type; 7-yr DFS was L=84.7% and P=81.3%. There were no significant interactions between treatment and stratification variables. 310 deaths occurred (L=164, P=146); there was no statistically significant difference in OS with L v P (HR=1.15, 95% CI 0.92, 1.44; P=0.22); 7-yr OS was L=91.8% and P=92.3%; 306 BCFI events occurred (L=127, P=179); L v P resulted in a statistically significant 29% decrease in BCFI events (HR=0.71, 95% CI 0.56, 0.89; P=0.003); 7-yr cumulative incidence (CIn) of BCFI was L=6.7% v P=10%; 175 DRs occurred (L=73, P=102); L v P resulted in a statistically significant 28% reduction in DR (HR=0.72, 95% CI 0.53, 0.97; P=0.03). There were 169 OF (L=91, P=78). There were no significant differences in time to OF with L v P (P=0.27). CIn of OF through 7 yrs was L=5.4% v P=4.8%. There were 127 AT events (L=69, P=58). Treatment with L did not result in an overall statistically significant increase in AT events compared to P (P=0.33). CIn of AT through 7 yrs was L=3.9% v P=3.3%.

Conclusions: After 5 yrs of an AI or TAM→AI, the beneficial effect of extended tx with 5 yrs of L on DFS did not reach statistical significance. There was no significant improvement in OS with L but L provided a significant improvement in BCFI and DR.

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Publication Number: S1-07

Title: Immune sculpting of the triple negative breast cancer genome

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Body: Background: Tumors with infiltrating lymphocytes (TIL) demonstrate a better prognosis particularly in TNBC and HER2 positive breast cancer. Two competing hypothesis predict contrasting relationships of TILs and genomic heterogeneity. On one hand, a strong immune response may lead to “pruning” of intratumor heterogeneity by eliminating immunogenic clones resulting in a near equilibrium, hence better prognosis, while cancers that escape the surveillance may evolve towards greater clonal heterogeneity and genomic complexity. In some cancers, the predicted neoantigens are less frequent than expected by chance also suggesting immune mediated elimination of neoplastic clones (Rooney et al. 2015). Studies also showed an inverse association between immune cell infiltration and intratumor clonal heterogeneity (Morris et al. 2016). On the other hand, cancers with greater genomic instability and mutational burden will have larger clonal heterogeneity and therefore more neoantigens and greater immune infiltration. Indeed, a positive correlation between overall mutation load and immune activity in the tumor microenvironment was observed in pooled data across a broad range of cancer types (Brown et al. 2014, Rooney et al. 2015, Schumacher and Schreiber 2015).

Methods: We assessed these two competing hypothesis and examined the relationship between genomic complexity and immune gene expression in different breast cancer subtypes. We used previously described immune metagene expression (DNA microarray n=655) as measures of immune infiltration in the TCGA data set (RNA-Seq n=1215). We compared somatic mutations, mutation count, neoantigen load, clonal heterogeneity metrics and the distribution of mutations in 119 canonical cancer genes and 12 cancer pathways between good and poor prognosis TNBC (n=208) corresponding to high and low immune infiltration.

Results: A positive but weak correlation between mutation count and immune metagene expression was observed when all breast cancer subtypes were analyzed together ($P=0.08$). This was driven by the generally higher mutation count and immune infiltration in TNBC. When TNBC was analyzed separately, good prognosis TNBC with high immune infiltration had lower total mutation count ($P=0.021$) and predicted neo-antigen count ($P=0.035$). Clonal heterogeneity was also lower in good prognosis TNBC ($P=0.001$). There was a strong inverse relationship of dispersion in mutation variant allele frequencies and immune metagene expression. CASP8 was the top enriched mutation in TNBC with high immune infiltration ($P=0.007$ with no adjustment for multiple testing).

Conclusions: High immune infiltration is associated with reduced intratumor heterogeneity in TNBC suggesting immune sculpting of the tumor and a near equilibrium between the cancer and immune surveillance. Surgical resection of the primary tumor may tilt the balance towards the immune system resulting in the better prognosis of high-TIL TNBC. TNBC with low immune infiltration has greater clonal heterogeneity and mutation load and may represent the consequence of escape from immune surveillance. Mutation of CASP8 may be one way to evade tumor cell killing in high-TIL TNBC as previously noted.

2016 San Antonio Breast Cancer Symposium

Publication Number: S1-08

Title: Prognostic associations of tumor-infiltrating lymphocytes (TIL) in metastatic HER2-positive breast cancer (BC) treated with trastuzumab and pertuzumab: A secondary analysis of the CLEOPATRA study

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Body: Background

The presence of stromal TILs (sTILs) is associated with a better prognosis with anti-HER2 therapy in primary HER2-positive BC. The prognostic value of TILs in the advanced setting with pertuzumab-based therapy is unknown.

Methods

The CLEOPATRA trial randomly assigned 808 patients with metastatic HER2-positive BC to receive pertuzumab or placebo in combination with trastuzumab and docetaxel. We evaluated %TILs using our previously described method. For concordance evaluation, 40 slides from metastatic samples were independently analysed by two pathologists. TILs were examined for associations with clinicopathological factors, progression-free survival (PFS), overall survival (OS), and treatment interactions using Cox regression models fitting sTILs as a continuous variable (per 10%) adjusting for treatment arm, age, estrogen receptor (ER) status, *PIK3CA* genotype, and visceral vs. non-visceral disease at screening.

Results

Tumour samples from 678 (84%) participants were available. 519 (76.5%) were archival and 155 (22.9%) were obtained fresh, ≤ 45 days prior to study treatment start. Median follow-up for OS was 50 months, with 519 PFS events and 358 deaths. 54% of patients were treatment naïve i.e. had not received prior chemotherapy nor trastuzumab. The median sTIL level was 10% (1-95%). sTIL evaluation was highly concordant between pathologists ($R=0.93$). Fresh vs. archival samples had significantly lower sTILs (10% vs 15%, $p=0.0004$). sTIL levels significantly differed by ethnicity (15% Asians, 10% white, 5% African-Americans, $p=0.0007$), but not age ($p=0.26$). Higher sTILs were observed in ER-negative vs. ER-positive tumors (15% vs 10%, $p<0.001$).

In the whole cohort for PFS, higher sTIL levels trended towards a better outcome independent of treatment (adjusted HR:0.95, 95%CI:0.90-1.00, $p=0.06$). For OS, the prognostic effect of sTILs reached statistical significance, with each 10% increase in sTILs associated with an 11% reduction in the risk of death (adjusted HR:0.89, 95%CI:0.83-0.96, $p=0.001$). The prognostic effect was observed independent of treatment arm, ER status, *PIK3CA* genotype, prior treatment or presence of visceral disease at screening, and in both fresh and archival tissue samples.

There was no significant interaction (int) between pertuzumab and sTILs for PFS (Pint=0.4) nor OS (Pint=0.6). There were no significant interactions between pertuzumab and sTILs for OS in subgroups of *PIK3CA* mutated (Pint=0.2) or *PIK3CA* WT (Pin=0.2), nor treatment naïve (Pint=0.3) vs prior treatment (Pint=0.5).

The 5-year estimates of OS according to median $\leq 10\%$ vs $>10\%$ sTILs in the placebo arm were 26% (95%CI:19-37) vs. 39% (95%CI:32-48), while in the pertuzumab arm 42% (95%CI:33-53) vs. 56% (95%CI:47-66) respectively.

Conclusion

In advanced HER2-positive disease, sTILs are still evident, though at lower levels, but are nevertheless significantly associated with prognosis, with effects stronger for OS than PFS. This suggests that the influence of anti-tumour immunity persists in the advanced first line setting and that enhancement by immunotherapeutic approaches could potentially further improve survival.

2016 San Antonio Breast Cancer Symposium

Publication Number: S1-09

Title: Evaluation of tumor-infiltrating lymphocytes (TILs) as predictive and prognostic biomarker in different subtypes of breast cancer treated with neoadjuvant therapy - A metaanalysis of 3771 patients

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Body: Background: Tumor-infiltrating lymphocytes (TILs) have been shown to be predictive for response to neoadjuvant therapy, in particular in triple-negative and HER2 positive breast cancer, suggesting that subtypes of breast cancer are immunogenic. The role of TILs in luminal breast cancer as well as the impact on prognosis in the different subtypes is less clear. In this study we evaluated TILs in a total of 3771 breast carcinomas from 6 prospective neoadjuvant clinical trials and evaluated their relevance for pCR, DFS and OS in different molecular subtypes.

Methods: A total of 3771 tumors from the clinical studies GeparDuo, GeparTrio, GeparQuattro, GeparQuinto, GeparSixto, GeparSepto were evaluated for stromal TILs by standardized methodology. Data on pCR were available for all tumors, DFS and OS was available for 2560 tumors. Logistic regressions, Cox regressions and Kaplan-Meier analyses were performed. In addition, a combined analysis of pCR, survival and TILs in different subtypes was performed.

Results: In the complete cohort of 3771 tumors, increased TILs ($\geq 60\%$) were observed in 19% of tumors, these tumors with $\geq 60\%$ TILs had a pCR rate of 44% ($p < 0.0005$). Increased stromal TILs ($\geq 60\%$) were observed in 30% of TNBC ($n=906$), in 19% of HER2+ tumors ($n=1379$) and in 13% of HR+/HER2- tumors ($n=1366$). In all three subtypes, increased TILs were significantly associated with increased pCR rates ($p < 0.0005$). In univariate logistic regression analysis of stromal TILs as a continuous variable, a 10% increase in TILs increased the probability to achieve a pCR by 16% in TNBC (OR 1.16), 13% in HER2+ (OR 1.13) and 33% in HR+/HER2- (OR 1.31, $p < 0.0005$ for all three groups). Similar results were observed in multivariate logistic regression ($p < 0.0005$ for all three groups). In univariate Cox regression, increased TILs were associated with improved DFS survival in TNBC (HR 0.93 per 10% TILs, $p=0.01$) and HER2+ BC (HR 0.93 per 10% TILs, $p=0.02$). In luminal (HR+/HER-) tumors there was no effect of TILs observed on DFS ($p=0.46$). In the luminal group increased TILs were associated with reduced OS (HR 1.10 per 10% increase in TILs, $p=0.01$), and increased TILs ($\geq 60\%$) were associated with worse survival in Kaplan-Meier analysis in luminal tumors (DFS: $p=0.04$, OS: $p < 0.0005$). A detailed analysis of subgroups of luminal BC as well as the link to pCR will be presented.

Conclusion: Our results suggest that tumor-infiltrating lymphocytes are a strong predictive marker for response to neoadjuvant chemotherapy in all molecular subtypes, and this predictive effect is translated into a survival benefit in HER2+ BC and TNBC. In contrast, a survival benefit is not observed in luminal BC, suggesting a different biology of the immunological infiltrate in this subtype.

2016 San Antonio Breast Cancer Symposium

Publication Number: S1-10

Title: Clinical implications of somatic mutations in post-menopausal early-stage estrogen receptor (ER)-positive HER2-negative breast cancer (BC): Results from the BIG 1-98 study

Loi S, Asher R, Lee CK K, Luen S, Savas P, Kammler R, Dell'Orto P, Blasi OM M, Demanse D, JeBailey L, Dolan S, Hackl W, Thuerlimann B, Viale G, Regan M and Colleoni MA A. Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia; NHMRC Clinical Trials Centre, University of Sydney, Sydney, NSW, Australia; International Breast Cancer Study Group (IBSCG), Bern, Switzerland; European Institute of Oncology, Milan, Italy and Novartis Institute for BioMedical Research, Cambridge, MA.

Body: Background

Next generation sequencing (NGS) has revealed that ER+/HER2- BCs have diverse somatic copy number and mutation profiles but thus far the clinical relevance of such findings is unknown. We characterized the molecular alterations in post-menopausal primary BC patients treated in the BIG 1-98 adjuvant letrozole and tamoxifen study and evaluated their associations with prognosis.

Materials and Methods

NGS was used to genotype DNA from archival primary tumor blocks for 286 cancer-related genes. From 2706 available eligible samples (confirmed ER+, excluding HER2-positivity or neoadjuvant treatment, adequate DNA quality/quantity), a case-cohort design selected 764 samples: all distant relapses and a stratified sampling of non-relapses after 8yr median follow-up. Mutation prevalence and associations with clinicopathological factors (CP) as well as distant recurrence-free interval (DRFS) were analyzed using weighted tests and Cox regression models, with sampling weights to represent the ER+/HER2-negative trial population. Multivariable analyses were adjusted for tumor size, nodal status, grade and age.

Results

NGS data was available from 538/764 samples (70%), there were 140 (26%) distant relapses. Median sequencing depth was 483x. There was a mean of 11 mutations (1-46) per sample, with 25% having 13 or more mutations and no tumors without a mutation.

Overall 28 genes were altered at a frequency of >10%. The most commonly mutated genes were *PIK3CA* (49.3%), *NCOR1* (27.2%), *MAP3K1* (23.8%) *TP53* (16.6%), *CCND1* (17.8%) and *GATA3* (17.1%).

Alterations that were significantly associated with both Luminal B (Ki67 >14%) and grade 3 included *TP53* mutations (p<0.001), *FGFR1* (p=0.001) and *MYC* amplifications (p=0.004).

Gene alterations that were significantly associated with shorter DRFS included *TP53* (HR:2.16), *ARID1A* (HR:2.43), *CHEK2* (HR:2.54), *BRCA2* (HR:1.93), *PTEN* (HR:2.03), *CCND1* (HR:1.82) and *FGFR1* (HR:1.78). *PIK3CA* was significantly associated with lower risk of distant relapse (HR:0.64; 0.43-0.97). Increasing number of total mutations was significantly associated with shorter DRFS (HR:1.04; 95% CI 1.01-1.07; p=0.006). In the multivariable model adjusted for CP factors, *ARID1A*, *BRCA2*, *CCND1*, *CHEK2* and *PTEN* remained independent for shorter DRFS.

Greater than 90% of *PIK3CA* mutations co-existed with another alteration, most common being *NCOR1* (29%), *MAP3K1* (24%), *CDH1* (16%), *GATA3* (16%), *TP53* (14%) and *CCND1* (13%). Patients with a *PIK3CA* mutation had greater benefit with letrozole over tamoxifen monotherapy (HR 0.32; 0.13-0.8) than those without (HR:0.70; 0.33-1.48) (Pint=0.06). This effect was strongest in the subgroup of *PIK3CA* mutant patients who were *CCND1* and *TP53* wild-type (HR:0.24, 0.12-0.48; Pint=0.02) with only 1% relapsing at 5 years.

Conclusion:

For the first time, we report the prognostic relevance of oncogenic mutations in ER+/HER2- postmenopausal early-stage BCs from a clinical trial. Tumors with *PIK3CA* mutations derived greater benefit from letrozole over tamoxifen monotherapy, especially if wild-type for *CCND1* and *TP53*. These findings could significantly improve prognostic risk classification and guide future clinical trials of targeted therapies in ER+/HER2- BCs.

2016 San Antonio Breast Cancer Symposium

Publication Number: S2-01

Title: Breast cancer risks associated with mutations in cancer predisposition genes identified by clinical genetic testing of 60,000 breast cancer patients

Couch FJ J, Hu C, Lilyquist J, Shimelis H, Akinhanmi M, Na J, Polley EC C, Hart SN N, McFarland R, LaDuca H, Huether R, Goldgar DE E and Dolinsky JS S. Mayo Clinic, Rochester, MN; Ambry Genetics, Aliso Viejo, CA and University of Utah, Salt Lake, UT.

Body: Clinical genetic testing panels are broadly used to gather information about cancer predisposition in individuals with personal and/or family history of breast cancer. However, the involvement of several of the genes on clinical testing panels in predisposition to breast cancer, such as *MRE11A* and *RAD50*, has recently come into question. In addition, accurate risk estimates for breast and other cancer are not well defined for the majority of genes on testing panels. We studied 60,000 women diagnosed with breast cancer who were tested for germline cancer predisposing mutations using hereditary cancer gene panels. Information on personal and family cancer history, age of diagnosis, and ethnicity of patients was obtained from test requisition forms. Greater than 90% met National Comprehensive Cancer Network HBOC testing criteria. To estimate gene-specific risks for breast cancer, case-control analyses were performed comparing the frequencies of pathogenic mutations from Caucasian cancer cases with frequencies from Caucasian, non-Finnish, non-TCGA controls from the Exome Aggregation Consortium (ExAC) database. Mutations were detected in 9% of breast cancer patients. Twelve genes displayed a significant association ($p < 0.05$) with breast cancer. Nine of these genes, including *ATM*, *RAD51D*, *NF1*, and *MSH6*, were associated with moderate risk ($RR > 2.0$) of breast cancer and three genes (*BRCA1*, *BRCA2*, *PALB2*) were associated with high risk ($RR > 5.0$) of breast cancer. Cumulative age-dependent risk models were developed for each gene. This large clinical testing dataset of 60,000 women with breast cancer provides useful data for many predisposition genes previously lacking risk estimates, and should prove useful for clinical risk management of patients with inherited mutations in these genes.

2016 San Antonio Breast Cancer Symposium

Publication Number: S2-02

Title: The landscape of somatic genetic alterations in BRCA1 and BRCA2 breast cancers

Burke KA A, Macedo GS S, Piscuoglio S, Ng CK K, Geyer FC C, Martelotto LG G, Papanastasiou AD D, De Filippo MR R, Schultheis AM M, Brogi E, Robson M, Wen YH, Weigelt B, Schnitt SJ J, Tung N and Reis-Filho JS S. Memorial Sloan Kettering Cancer Center, New York, NY and Beth Israel Deaconess Medical School, Boston, MA.

Body: Introduction: Women carrying *BRCA1* or *BRCA2* germline mutations have a 45-80% lifetime risk of developing breast cancer (BC). *BRCA1* and *BRCA2* are perceived as bona fide tumor suppressor genes, whereby bi-allelic inactivation in tumor cells is required for tumorigenesis. Recent studies have indicated that loss of heterozygosity (LOH) of the wild-type allele of *BRCA1* may be heterogeneous and constitute a late event. Therefore, additional somatic events prior to full BRCA1/2 inactivation may be required for tumorigenesis. Given that the somatic events that result in the development of BRCA1/2-BCs and their chronology are not understood, here we sought to define the genomic landscape of BRCA1/2-BCs and whether LOH of *BRCA1/2* wild-type allele and/or mutations affecting additional tumor suppressor genes would be clonal or subclonal in these cancers.

Methods: We retrieved 29 BRCA1-BCs and 10 BRCA2-BCs from the Pathology Departments of the authors' institutions. DNA extracted from microdissected tumor and normal breast samples was subjected to targeted capture massively parallel sequencing using either the MSK-IMPACT assay or an assay targeting all exons of 254 genes recurrently mutated in BC or related to DNA repair. Somatic single nucleotide variants, small insertions and deletions and copy number alterations affecting genes present in both sequencing assays (111 genes) were defined using state-of-the-art bioinformatics algorithms. ABSOLUTE and FACETS were employed to define clonal (i.e. present virtually in 100% of the cancer cells of a given case) and subclonal mutations and the presence of LOH of the BRCA1 and BRCA2 wild-type alleles.

Results: Our analysis revealed bi-allelic inactivation of *BRCA1* in 28 of 29 BRCA1-BCs (93% harbored LOH of the *BRCA1* wild-type allele and 3% harbored a second somatic *BRCA1* pathogenic mutation). The only BRCA1-BC lacking bi-allelic inactivation of *BRCA1* was an estrogen receptor-positive lobular carcinoma, lacking genomic features consistent with homologous recombination DNA repair defects, diagnosed at 62 years of age. Bi-allelic inactivation of *BRCA2* was found in all cases (100% of harbored LOH of the *BRCA2* wild-type allele). A clonal somatic 'second hit' resulting in bi-allelic inactivation of *BRCA1* or *BRCA2* was detected in 76% and 100% of BRCA1-BCs and BRCA2-BCs, respectively. In BRCA1-BCs, *TP53* mutations were detected in 76% of cases, and these mutations were found to be clonal in 58% of cases. The repertoire of somatic mutations affecting BRCA1-BCs included clonal somatic mutations or homozygous deletions of known tumor suppressor genes, such as *PTEN*, *RB1*, *CDKN2A* and *NF1*. In contrast, only 10% of the BRCA2-BCs harbored *TP53* somatic mutations. Though clonal somatic mutations in several cancer genes were detected, 40% of BRCA2-BCs had no mutations affecting the cancer genes analyzed.

Conclusions: Bi-allelic inactivation of *BRCA1* and *BRCA2* are frequent events in BRCA1-BCs and BRCA2-BCs, respectively. In a subset of BRCA1-BCs, however, the second 'hit' appeared to be subclonal, whereas mutations affecting *TP53* and other tumor suppressor genes were clonal, supporting the notion that at least in a subset of these tumors, loss of the wild-type allele of *BRCA1* may be preceded by inactivation of another tumor suppressor gene.

2016 San Antonio Breast Cancer Symposium

Publication Number: S2-03

Title: Does BRCA status affect outcome in young breast cancer patients? Results from the prospective study of outcomes in sporadic and hereditary breast cancer (POSH)

Eccles DM M, Copson ER R, Maishman T, Tapper W, Cutress R, Gerty S, Stanton L, Altman DG G, Durcan L, Simmonds P, Decker B, Allen J, Luccarini C, Easton D, Dunning A and POSH Steering Group and Collaborators. Cancer Sciences Academic Unit and Southampton Clinical Trials Unit, Faculty of Medicine, University of Southampton and University Hospital Southampton Foundation Trust, Southampton, United Kingdom; Centre for Statistics in Medicine, University of Oxford, Oxford, United Kingdom; Strangeways Research Laboratories, Cambridge University, Cambridge, United Kingdom and POSH Steering Group and Collaborators.

Body: Background

Germline mutations in BRCA1/2 account for ~3% of breast cancer cases but >10% of young patients who present with triple negative (TN) breast cancer. Young age at diagnosis is also associated with an increased risk of recurrence and inferior survival compared to older patients. Numerous publications describe an increased incidence of adverse biological features in tumours from young breast cancer patients; however it is unclear whether these fully explain the poor outcome.

The effect of carrying a BRCA1/2 mutation on the prognosis of breast cancer remains controversial with retrospective studies reporting better, similar and worse outcomes for mutation carriers compared to patients with sporadic tumours. BRCA carriers could feasibly have enhanced or reduced sensitivity to certain chemotherapeutics; however retrospective studies are problematic due to missing data and biased ascertainment. POSH is multicentre prospective observational cohort study designed to investigate factors which affect prognosis in young breast cancer patients (Copson et al, JNCI, 2013). Here we report the pathology, treatment and outcome of patients with TN tumours as a preliminary analysis to determine the impact of a germline BRCA1 mutation on survival. The whole cohort analysis including BRCA1 and BRCA2 is in progress.

Methods

2956 patients aged ≤ 40 at breast cancer diagnosis were recruited from 127 UK oncology centres between 2000 and 2008. Patient characteristics, family history, risk factors, tumour pathology and treatment information, and blood DNA were collected at recruitment. Follow-up data were collected at 6 and 12 months, then annually. Summary statistics were used to describe patients by BRCA1 status. Kaplan-Meier estimates were used to describe univariate survival data.

Results

BRCA1 status is currently available for 542 patients with TN tumours. Pathogenic BRCA1 mutations were identified in 122 patients (BRCA1+); 420 had no BRCA1 mutation (BRCA1-). BRCA1+ were younger than BRCA1- (median age 34 vs 36 years, $p < 0.001$) and more likely to have a positive family history ($p < 0.001$). There were no significant differences between BRCA1+ vs BRCA1- for: median tumour size (20.8mm vs 23.0mm); tumour grade distribution (95.8% grade 3 vs 93.6%); nodal involvement (35.2% node positive vs 39.9%); or presence of metastases at diagnosis (0.0% vs 1.0%).

Median follow-up was 7.3 years. Overall survival of patients with stage 1-3 disease treated with anthracycline +/- taxane neoadjuvant chemotherapy (n=538; 151 deaths) was better for BRCA1+ vs BRCA1- (79.1% vs 73.6% at 5-yrs; HR[95%CI]=0.84[0.57,1.25], $p=0.388$). Distant disease-free survival (DDFS) was also higher for BRCA1+ (5-yr DDFS 76.1% vs 71.5%; HR[CI]=0.92[0.63,1.35], $p=0.682$). Moreover, survival after first distant relapse was better for BRCA1+ patients (41.9% vs 36.8% at 1-yr; HR[CI]=0.78[0.51,1.18], $p=0.233$).

Conclusions

Our prospective data show better survival in young BRCA1+ patients with early TN breast cancer treated with anthracycline/-taxane chemotherapy than BRCA1- patients. However, the difference between the groups was not significant in this partial sample. Results for the whole cohort will be available by the time of the meeting.

2016 San Antonio Breast Cancer Symposium

Publication Number: S2-04

Title: RANK ligand as a target for breast cancer prevention in *BRCA1* mutation carriers

Lindeman GJ J, Nolan E, Vaillant F, Branstetter D, Pal B, Giner G, Whitehead L, Lok SW W, Mann GB B, kConFab Consortium, Rohrbach K, Huang L-Y, Soriano R, Smyth GK K, Dougall WC C and Visvader JE E. The Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia; The Royal Melbourne Hospital, Melbourne, VIC, Australia; The Victorian Comprehensive Cancer Centre, Melbourne, VIC, Australia; The University of Melbourne, Melbourne, VIC, Australia; Amgen Inc, CA; The Royal Women's Hospital, Melbourne, VIC, Australia; kConFab, Australia and QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia.

Body: Background: *BRCA1* mutation carriers commonly undergo prophylactic mastectomy to reduce their risk of breast cancer. The precise role of chemoprevention with tamoxifen, which reduces the incidence of ER-positive breast cancer in the general population, is uncertain for *BRCA1* mutation carriers, where uptake has been modest. The identification of an effective and acceptable prevention therapy therefore remains a 'holy grail' for the field. Precancerous *BRCA1*^{mut/+} tissue harbors an aberrant population of luminal progenitor cells and deregulated progesterone signaling has been implicated in *BRCA1*-associated oncogenesis. Since Receptor Activator of Nuclear Factor-kappa B ligand (RANKL) is a key paracrine effector of progesterone signaling, and RANKL and its receptor RANK contribute to mammary tumorigenesis, we investigated a role for this pathway in the preneoplastic phase of *BRCA1* mutation carriers.

Methods: We explored a role for the RANK/RANKL pathway during the preneoplastic phase in freshly isolated (histologically normal) patient specimens from *BRCA1* mutation carriers using several approaches. RANK and RANKL expression in breast cancer was evaluated in formalin fixed paraffin embedded (FFPE) archival sections by IHC from the kConFab and the Amgen Tissue Banks. All samples were obtained with patient consent and relevant IRB approval. A role for RANKL inhibition in attenuating tumor onset was studied using the MMTV-cre/*Brca1*^{fl/fl}/p53^{+/-} mouse model that recapitulates human basal-like breast cancer.

Results: We identified two subsets of luminal progenitors (RANK⁺ and RANK⁻) in histologically normal tissue of *BRCA1* mutation carriers and found that RANK⁺ cells are highly proliferative, exhibit grossly aberrant DNA repair and bear a molecular signature similar to that of basal-like breast cancer. Moreover, high levels of RANK expression prevailed in established *BRCA1*-associated tumors. These data suggest that RANK⁺ and not RANK⁻ progenitors are a key target population in these women. Notably, inhibition of RANKL signaling by denosumab in 3D breast organoids derived from pre-neoplastic *BRCA1*^{mut/+} tissue attenuated progesterone-induced proliferation. Furthermore, inhibition of RANKL with either the RANKL inhibitor OPG-Fc or a RANKL monoclonal antibody in a *Brca1*-deficient mouse model significantly curtailed mammary tumorigenesis, when compared to controls (p<0.001).

Conclusions: Together these findings identify a targetable pathway in a putative cell of origin population in *BRCA1* mutation carriers and implicate RANKL blockade as a promising breast cancer prevention strategy.

2016 San Antonio Breast Cancer Symposium

Publication Number: S2-05

Title: Efficacy and tolerability of veliparib (V; ABT-888) in combination with carboplatin (C) and paclitaxel (P) vs placebo (Plc)+C/P in patients (pts) with *BRCA1* or *BRCA2* mutations and metastatic breast cancer: A randomized, phase 2 study

Han HS Sook, Diéras V, Robson ME E, Palácová M, Marcom PK Kelly, Jager A, Bondarenko I, Citrin D, Campone M, Telli ML L, Domchek SM M, Friedlander M, Kaufman B, Ratajczak C, Coates A, Bonnet P, Qin Q, Qian J, Giranda VL L, Shepherd SP P, Isakoff SJ J and Puhalla S. Moffitt Cancer Center, Tampa, FL; Institut Curie, Paris, France; Memorial Sloan Kettering Cancer Center, New York, NY; Masarykův Onkologický ústav, Brno, Czech Republic; Duke University, Durham, NC; Erasmus MC Cancer Institute, Rotterdam, Netherlands; Dnepropetrovsk City Hospital, Dnepropetrovsk, Ukraine; Midwestern Regional Medical Center, Zion, IL; Institut de Cancérologie de l'Ouest, Saint Herblain, France; Stanford University School of Medicine, Stanford, CA; University of Pennsylvania, Philadelphia, PA; Prince of Wales Hospital, Sydney, NSW, Australia; Sheba Medical Center, Tel Hashomer, Israel; AbbVie, Inc, Chicago, IL; Massachusetts General Hospital, Boston, MA and University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Body: Background: Poly(ADP-ribose) polymerase (PARP) inhibitors block DNA damage repair and may thereby enhance the clinical activity of DNA-damaging chemotherapy. Homologous recombination is defective in *BRCA1/2*-mutated tumors, leading to more error-prone mechanisms of DNA repair and increased sensitivity to PARP inhibition. V is a potent PARP inhibitor that enhances the antitumor activity of platinum agents in preclinical models. This phase 2 trial (NCT01506609) investigated the safety and efficacy of V+C/P or V+ temozolomide (TMZ) vs Plc+C/P in pts with locally recurrent or metastatic breast cancer harboring a *BRCA1* or *BRCA2* mutation. Results of the V+C/P and Plc+C/P arms are presented; V+TMZ results will be presented separately.

Methods: Pts ≥ 18 years with histologically confirmed locally recurrent or metastatic breast cancer were randomized 1:1:1 to: 1) V 40 mg BID D1–7+TMZ, 28-D cycle; 2) V 120 mg BID D1–7+C AUC 6, D3 and P 175 mg/m², D3, 21-D cycle; or 3) Plc BID D1–7+C/P. Key eligibility criteria included deleterious *BRCA1/2* mutation, ≤ 2 prior chemotherapies for metastatic disease, no prior platinum agent, and no CNS metastases. Randomization was stratified by hormone receptor status, prior cytotoxic therapy, and ECOG PS. The primary endpoint was progression-free survival (PFS) per RECIST 1.1 of each V arm vs Plc+C/P by independent review. Primary analysis occurred at the 112th PFS event in the V+C/P and Plc+C/P arms. Overall survival (OS), objective response rate (ORR), tolerability, and quality of life were also evaluated.

Results: A total of 196 pts (193 *BRCA+* per central lab) were randomized to receive double-blinded V+C/P (n=97) or Plc+C/P (n=99). Baseline demographics and disease characteristics were balanced across all treatment arms. Median study drug exposure was 10 cycles for Plc+C/P and 12 cycles for V+C/P. The V+C/P arm demonstrated numeric improvements for both PFS and OS compared to the Plc+C/P arm; improvement in ORR was statistically significant (Table 1). There was no meaningful increase of toxicity with addition of V. The most common treatment-emergent adverse events (AEs) with Plc+C/P or V+C/P were neutropenia (74%/74%), thrombocytopenia (70%/71%), and nausea (58%/71%). Grade ≥ 3 AEs in $\geq 30\%$ of pts were neutropenia (55%/56%) and thrombocytopenia (26%/31%), respectively. There was no difference in the use of G-CSF with addition of V. Significant improvements in fatigue, pain, and insomnia (all $P < 0.05$) were observed with V+C/P vs Plc+C/P.

Conclusions: This is the first randomized phase 2 trial of a PARP inhibitor in combination with platinum-based therapy for treatment of *BRCA1/2*-mutated advanced breast cancer. V+C/P demonstrated significantly higher ORR and symptom improvement compared to Plc+C/P, with nonsignificant trends for improved OS and PFS. Phase 3 trials are ongoing.

Table 1

Efficacy (ITT population – <i>BRCA</i> mutation)	Plc+C/P, n=98	V+C/P, n=95	HR (95% CI); <i>P</i> value
PFS (mo, 95% CI)	12.3 (9.3–14.5)	14.1 (11.5–16.2)	0.789 (0.536–1.162); 0.231
OS (mo, 95% CI)	25.0 (18.1–34.8)	28.5 (22.4–NR)	0.725 (0.468–1.121); 0.148
ORR, % (95% CI)	61.3 (49.7–71.9)	77.8 (66.4–86.7)	<i>P</i> =0.027

2016 San Antonio Breast Cancer Symposium

Publication Number: S2-06

Title: DNA repair deficiency biomarkers and MammaPrint high1/(ultra)high2 risk as predictors of veliparib/carboplatin response: Results from the neoadjuvant I-SPY 2 trial for high risk breast cancer

Wolf DM M, Yau C, Sanil A, Glas A, Petricoin C, Wulfkuhle J, Brown-Swigart L, Hirst G, I-SPY 2 TRIAL Investigators, Buxton M, DeMichele A, Hylton N, Symmans F, Yee D, Paoloni M, Esserman L, Berry D, Rugo H, Olapade O and van 't Veer L. University of California, San Francisco; Berry Consultants, LLC; Agendia, Inc; George Mason University; QuantumLeap Healthcare Collaborative; University of Pennsylvania; University of Texas, MD Anderson; University of Minnesota; University of Chicago and Equal Contribution.

Body: Background: The PARP inhibitor veliparib in combination with carboplatin (VC) was one of the experimental regimens evaluated in the phase 2 neoadjuvant I-SPY 2 standing trial for high risk breast cancer patients. VC graduated in the triple negative (TN) signature. However, not all TN patients achieved pathologic complete response (pCR) and some HR+HER2- patients responded. The I-SPY 2 biomarker component provides a platform for rigorous evaluation of mechanism-of-action-based markers in the context of established biomarkers (HR, HER2, MammaPrint) within the trial. Here, we report results from 5 investigator-submitted biomarker proposals and the MammaPrint High1/High 2 (MP1/2) classification as specific predictors of VC response.

Methods: Data from 116 HER2- patients (VC: 72 and concurrent controls: 44) were available. BRCA1/2 germline mutation was assessed by Myriad Genetics. 3 expression signatures relating to DNA damage repair deficiency (PARPi-7, BRCAness and CIN70) and MP1/2 classification were evaluated on Agilent 44K arrays. PARP1 levels were measured using reverse phase protein arrays. We used logistic modeling to assess biomarker performance. A biomarker is considered a specific predictor of VC response if it associates with response in the V/C arm but not the control arm, and if the biomarker x treatment interaction is significant (likelihood ratio test, $p < 0.05$). In an exploratory analysis, we combined successful biomarkers to *refine* the 'predicted sensitive' group and used Bayesian modeling to estimate the pCR rates of 'predicted sensitive' TN and HR+HER2- patients in each arm.

Results: BRCA1/2 germline mutation status associates with VC response, but its low prevalence in the control arm precludes further evaluation. Of the biomarkers evaluated, three (PARPi-7, BRCAness, and MP1/2) associate with response in the VC arm but not the control arm, and have biomarker x treatment interactions with $p < 0.05$ that retains significance upon adjusting for HR status. These signatures are only moderately correlated. When we combined the PARPi-7 and MP1/2 classifications using a simple voting scheme, the 40% of TN patients who are PARPi7-high *and* MP2 have an estimated pCR rate of 79% in the VC arm. In contrast, TN patients negative for at least one of these signatures (PARPi7-low *and/or* MP1) only have an estimated pCR rate of 35%. Only 9% of HR+/HER2- patients are PARPi7-high *and* MP2; but these patients also appear more responsive to VC with estimated pCR rates of 49%, compared to 13% in 'biomarker-negative' HR+HER2- patients.

Conclusion: If verified in a larger trial, PARPi7, BRCAness and MP1/2 signatures may help refine predictions of VC response, thereby improving patient care. Evaluation of the combined signature for patients treated with platinum-based chemotherapy is ongoing.

2016 San Antonio Breast Cancer Symposium

Publication Number: S2-07

Title: Sentinel node detection after neoadjuvant chemotherapy in patient without previous axillary node involvement (GANEA 2 trial): Follow-up of a prospective multi-institutional cohort

Classe J-M, Loaec C, Alran S, Paillocher N, Tunon-Lara C, Gimbergues P, Faure-Virelizier C, Chauvet M-P, Lasry S, Dupre P-F, Verhaeghe J-L, De Blaye P, Gutowski M, Barranger E, Lecuru F, Lefevre Lacoeyille C, Loussert L, Lambaudie E, Ferron G and Champion L. Institut Cancérologie de l'Ouest Centre Gauducheau, Saint Herblain, France; Institut Curie, Paris, France; Institut Cancérologie de l'Ouest Centre Papin, Angers, France; Institut Bergonié, Bordeaux, France; Centre Jean Perrin, Clermont Ferrand, France; Centre Leon Berard, Lyon, France; Centre Oscar Lambret, Lille, France; Institut Curie, Saint Cloud, France; Centre Hospitalier Universitaire Morvan, Brest, France; Institut de Cancerologie de Lorraine, Nancy, France; Centre Hospitalier Les Oudairies, La Roche sur Yon, France; Centre Val d'Aurelle, Montpellier, France; Centre Lacassagne, Nice, France; Centre Hospitalier Europeen Pominou, Paris, France; Centre Hospitalier Universitaire, Angers, France; Centre Paul Stauss, Strasbourg, France; Institut Paoli Calmettes, Marseille, France and Institut Universitaire de Cancerologie Claudius Regaud, Toulous, France.

Body: Background

Half of the patient treated with neoadjuvant chemotherapy (NAC) for a large operable breast cancer has no axillary lymph node involvement at the time of surgery. Sentinel lymph node detection (SLND), after NAC, is aimed to select patient who should be safely spared of an axillary lymphadenectomy (ALND). GANEA 2 is a French prospective multi institutional trial, aimed to assess SLND after NAC.

Objective

To assess the risk of relapse for patients without previous axillary node involvement treated with NAC followed with a SLND without a systematic lymphadenectomy.

Patients and Method

Inclusion: FIGO stage T1-T3 infiltrating breast carcinoma, indication of NAC.

Exclusion: inflammatory cancer, local relapse, contra-indication to NAC, NAC interrupted due to progressive disease.

Design: indication to plan a NAC, axillary sonography with fine needle cytology before NAC to select patients without lymph node involvement, SLND after NAC. ALND was mandatory in case of SLN involvement (macro or micro-metastasis) or SLND failure.

Follow-up was scheduled with a medical visit / 6 months with axillary assessment and a mammography each year. Follow-up results are updated every 6 months.

Pathological analysis were carried out according to standard methods and classified according to the last American Joint Committee staging system.

Studied parameters were SLND detection rate, pathological results on breast specimen and nodes, rate of relapse (axilla, breast, metastasis), and survival.

Results

From July 2010 to February 2014, 587 patients were enrolled, from 17 institutions, and experienced breast tumor surgery and a SLND after NAC.

Each patient experienced breast surgery. A breast tumour pathological complete response was found in 21.3% (125/587).

SLND rate was 97% (570/587), with a median number of 2 sentinel nodes (1-9).

Patients with a sentinel detection failure (n=17) experienced a systematic lymphadenectomy, without any involvement (n=13), a micro-metastasis (n=2) and a macro-metastasis (n=2).

A total of 140 patients had at least one sentinel node involved: macro-metastasis (n=86), micro-metastasis (n=54). A lymphadenectomy was performed in 128 cases: metastasis free (n=100), macro-metastasis (n=17), micro-metastasis (n=11).

A total of 430 patients had a SLN metastasis free (75% ;430/570). A not mandatory lymphadenectomy was performed (n=14): metastasis free (n=11), macro-metastasis (n=2) and micro-metastasis (n=1). 17 patients were lost to follow-up.

A total of 399 patients without sentinel node involvement were followed 2.3 years (from 0.5 to 5.6 yrs). At 3 years overall survival was 97.8% [94.9-99.1], disease free survival was 94.8% [91.0-97.1%]. Six patients died. Fifteen patients experienced a relapse: 8 metastasis, 4 homolateral breast, 2 controlateral breast, 1 homolateral axillary relapse.

Conclusion

This is the most important series of patients followed 2.3 years after SLND without axillary lymphadenectomy after NAC for an advanced breast cancer, showing acceptable results. The current series validate the safety of this conservative strategies avoiding systematic lymphadenectomy to patients without initially involved axillary node treated with NAC.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-01

Title: A phase II, open-label, multicenter, translational study for biomarkers of eribulin mesylate: Evaluation of the utility of monitoring epithelial-to-mesenchymal transition (EMT) markers on tumor cells in the malignant plural effusion of patients with metastatic breast cancer (EXPECT-study)

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Body: Background: Epithelial-mesenchymal transition (EMT) is considered a possible mechanism of distant metastasis or resistance of cancer cells to anticancer drugs. Several reports suggest that eribulin methylate (eribulin) promotes mesenchymal-epithelial transition (MET) both in vitro and in vivo and have shown its inhibition of distant metastasis of engrafted carcinoma. However, no reports have examined the effects of eribulin in a clinical setting. The EXPECT-study investigates EMT/MET markers in metastatic breast cancer (MBC) patients using pleural effusion as a method of liquid biopsy.

Objectives: In MBC patients with malignant pleural effusion, we attempted to establish EMT/MET markers using the cancer cells in the pleural effusion, to assess the efficacy and safety of eribulin monotherapy, and to verify the EMT/MET markers as biomarkers in eribulin monotherapy.

Study design and eligibility criteria: The EXPECT study is a multicenter, open-label, single-arm phase 2 translational study. Eribulin will be administered at 1.4 mg/m² on Days (D) 1 and 8 for a 3-week cycle (C), and to obtain the cancer cells from the pleural effusion before and after eribulin administration, paired chest drainage will be performed on C1D1 and C1D8. MBC patients, regardless of their subtype, with pleural effusion are eligible for enrollment, and eribulin monotherapy in the study will be administered based on clinical practice. Therefore, there are no special inclusion/exclusion criteria except for the requirement that all patients be naïve to eribulin.

Methods: Spun-down cancer cells in the plural effusion will be processed via the cell block method and embedded in paraffin. An immunohistochemical analysis will be performed using candidate antibodies for EMT/MET markers, such as E-cadherin, cytokeratin (CK) 19, and 34betaE12 (CK1, CK5, CK10, CK14) as epithelial markers and N-cadherin, vimentin, Snail, Slug, Twist-1, ZEB1, ZEB2, ALDH1, and HIF-1alpha as mesenchymal markers, which are all concurrently being assessed in another confirmatory study. The changes in EMT/MET marker expression between pre- and post-treatment will be measured, and the relationship between these changes and the clinical outcome, such as the clinical benefit rate (CBR), progression-free survival, and overall survival, will be assessed.

Present and target accrual: The study was just approved by the institutional review board in March 2016 and is open for enrollment. The target accrual is 48 patients total, or 4 patients per month. The sample size calculation was not based on statistical consideration, such as power or type I error. As an illustration for the analysis, the relationship between the changes in the EMT/MET markers and the clinical outcome may be investigated by comparing the CBRs in the two groups with substantial and small changes in the marker expression. If we demonstrate CBRs of 38% and 13% in each group, respectively, then statistical significance will be demonstrated with a 0.10 significance level (2-sided).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-02

Title: A phase II trial of neoadjuvant docetaxel/cyclophosphamide followed by epirubicin/cyclophosphamide for triple-negative breast cancer

Tanaka N, Hirano A, Inoue H, Ogura K, Hattori A, Jibiki N, Yukawa H, Matsuoka A, Kodera A, Kamimura M, Naritaka Y and Shimizu T. Tokyo Women's Medical University Medical Center East, Tokyo, Japan; Tokyo Women's Medical University Yachyo, Medical Center, Yachiyo, Japan and Tokyo Women's Medical University Medical Center East, Tokyo, Japan.

Body: Background

Currently, an anthracycline followed by a taxane as adjuvant or neoadjuvant chemotherapy is considered the most effective treatment for patients with triple-negative breast cancer. However, docetaxel followed by an anthracycline as neoadjuvant chemotherapy results in a higher rate of pathological complete response (pCR) than the regimen including an anthracycline followed by docetaxel (Iwata et al. Jpn J Clin Oncol 2011). Adjuvant docetaxel/cyclophosphamide (DC) treatment resulted in prolonged survival compared to adjuvant doxorubicin/cyclophosphamide. Therefore, we planned a phase II trial of DC followed by epirubicin/cyclophosphamide (EC) (Trial registration: UMIN000011031).

Trial design

This is a phase II trial to evaluate the efficacy and toxicity of DC followed by EC as neoadjuvant therapy for triple-negative breast cancer. Patients will receive four cycles of docetaxel (75 mg/m²) and cyclophosphamide (600 mg/m²) every 21 days, followed by four cycles of epirubicin (90 mg/m²) and cyclophosphamide (600 mg/m²) every 21 days.

Eligibility criteria

Patients with histologically diagnosed triple-negative breast cancer, T1–4, N1–3, or T2–T4, N0 based on a core needle biopsy, will be included in this trial. Eligible patients must be between 20 and 70 years of age with a performance status of 0–2 and adequate organ function. They must not have undergone any prior operation, radiation therapy, chemotherapy, endocrine therapy, or immunotherapy.

Specific aims

The primary endpoint is the pCR rate in the breast and axilla, and the secondary endpoints are the breast-conserving rate, toxicities, feasibility, and 5-year overall survival and relapse-free survival. pCR is defined as disappearance of invasive cancer cells, including those in the axilla, although the presence of residual intraductal cancer is acceptable.

Statistical methods

The sample size was calculated using the Simon method, with a type I error of 10% and a study power of 80%. The expected pCR rate is 25%, with a threshold pCR rate of 10%, and the required number of patients has been estimated to be 33.

Present and target accrual

Patient accrual within two medical centers was started in September 2013. We plan to enroll a total of 34 patients in the trial.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-03

Title: A phase II trial of neoadjuvant epirubicin/cyclophosphamide followed by weekly nanoparticle albumin-bound paclitaxel with trastuzumab for HER2-positive breast cancer

Kodera A, Hirano A, Inoue H, Ogura K, Hattori A, Sakaguchi S, Yukawa H, Matsuoka A, Tanaka N, Kamimura M, Jibiki N, Fujibayashi M, Naritaka Y and Shimizu T. Tokyo Women's Medical University Medical Center East, Tokyo, Japan; Tokyo Women's Medical University Yachyo, Medical Center, Yachiyo, Japan; Tokyo Women's Medical University Medical Center East, Tokyo, Japan and Tokyo Women's Medical University Medical Center East, Tokyo, Japan.

Body: Background

Paclitaxel (PTX) is a standard treatment for metastatic breast cancer (MBC) and it is often used as adjuvant and neoadjuvant chemotherapy for patients with early-stage disease. Nanoparticle albumin-bound (Nab)-PTX was also effective in patients with metastatic and early-stage. A comparison of weekly and triweekly nab-PTX regimens suggested that weekly nab-PTX resulted in superior progression-free survival. However, the optimal dose and schedule of weekly nab-PTX have not been determined. The efficacy and tolerability of epirubicin/cyclophosphamide (EC) followed by weekly nab-PTX (125 mg/m²) ± trastuzumab in node-positive breast cancer was determined in our previous trial. A high pathologic complete response (pCR) rate was obtained in HER2-positive patients. However, because nab-PTX administration was frequently postponed and discontinued, the optimal dose needs to be determined. In the previous trial, the median relative dose intensity of nab-PTX was 80% among patients with pCR. Therefore the dose of nab-PTX was reduced by 20% in this newly designed trial.

Trial design

This phase II trial aimed to evaluate the efficacy and toxicity of neoadjuvant EC followed by weekly nab-PTX with trastuzumab in patients with HER2-positive breast cancer. Patients will receive 4 cycles of epirubicin (90 mg/m²) and cyclophosphamide (600 mg/m²) every 3 weeks, followed by 4 cycles of nab-PTX (100 mg/m²) on days 1, 8, and 15, over a 28-day cycle. Fifteen cycles of trastuzumab (2 mg/kg, loading dose: 4 mg/kg) will be added to the nab-PTX regimen.

Eligibility criteria

Surgery and chemotherapy-naïve patients with pathologically confirmed T2-4 N0-3 invasive breast cancer, as diagnosed by core needle biopsy, are included. Eligibility criteria include age 20–70 years, a performance status of 0–2, and adequate organ function.

Specific aims

The primary endpoint is the pCR rate in the breast and axilla. Secondary endpoints include the breast conservation rate, toxicities, relative dose intensities, feasibility, and overall survival. A pCR is defined as the disappearance of invasive cancer cells, including in the axilla; residual intraductal cancer is acceptable.

Statistical methods

The sample size was calculated using the Simon method, with a type I error of 5% and a study power of 80%. The expected rate of pCR is 72% with a threshold pCR rate of 45%. The required number of patients was estimated to be 25.

Present and target accrual

Patient accrual within two medical centers began in May 2014. A final study population of 30 patients is expected (Trial registration: UMIN000013886).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-04

Title: VENTANA (SOLTI-1501): Oral metronomic vinorelbine combined with endocrine therapy in luminal/HER2-negative early breast cancer: A window of opportunity trial

Adamo B, Vidal M, Gomez Pardo P, Zaragoza K, Ciruelos E, Virizuela JA Antonio, Blanch Tormo S, Pérez-Fidalgo JA, Murillo L, Lopez-Gonzalez A, Amillano Parraga K, Martinez Jañez N, González Farré X and Prat A. Hospital Clínic de Barcelona, Barcelona, Spain; SOLTI Breast Cancer Research Group, Barcelona, Spain; Hospital Universitari Vall d'Hebron, Barcelona, Spain; Hospital Universitario 12 de Octubre, Madrid, Spain; Clínica Quirón Sagrado Corazón, Sevilla, Spain; Fundación Instituto Valenciano de Oncología, Valencia, Spain; Hospital Clínico Universitario de Valencia, Valencia, Spain; Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain; Hospital de León, León, Spain; Hospital Universitari Sant Joan de Reus, Reus, Tarragona, Spain; Hospital Universitario Ramón y Cajal, Madrid, Spain; Hospital Universitari General de Catalunya, Sant Cugat del Vallés, Barcelona, Spain and Translational Genomics and Targeted Therapeutics in Solid Tumors, IDIBAPS, Barcelona, Spain.

Body: BACKGROUND

The CDK4/6 inhibitor palbociclib, in combination with endocrine therapy (ET), has been approved for patients (pts) with HR+/HER2- metastatic breast cancer (BC), suggesting that inhibition of the cell cycle in combination with ET is a strategy to keep exploring. In this context, vinorelbine (VNB) inhibits chromosome segregation during mitosis and blocks cells at G2/M. Interestingly, several metronomic schedules of VNB are being used in the clinical setting, a strategy that might not only affect cell-cycle but also aims to target tumor angiogenesis.

VENTANA is a “window-of-opportunity” trial designed to explore whether, similarly to CDK4/6 and mTOR inhibitors, oral metronomic VNB in combination with endocrine therapy induces a superior anti-proliferative effect than ET alone, as suggested by preclinical and clinical studies. We believe that a biological synergy of the combined treatment could open the door to include this treatment strategy in pts with BC as an alternative to CDK4/6 inhibitors.

METHODS

VENTANA is a phase 0 multicenter, three-arm, randomized clinical trial of oral metronomic VNB and letrozole (LET) versus either treatment alone in postmenopausal women with newly diagnosed, untreated HR+ and HER2-, stage I-III operable BC. Other eligibility criteria include primary tumor size ≥ 1 cm (cT1-3) and N0-1, ECOG PS 0-1 and evaluable diagnostic tumor sample. Pts are randomized (1:1:1) to receive LET 2.5mg daily, oral VNB 50mg 3 days a week, or LET 2.5mg daily and oral VNB 50mg 3 times a week. After 3 weeks of treatment, pts will undergo surgery, and both pre-treatment and post-treatment surgical samples will be analyzed for gene expression. Primary objective is to test if oral metronomic VNB and LET induce a superior anti-proliferative effect than either drug alone in pts with early BC defined as Luminal by PAM50. This will be evaluated by the expression of 11 proliferative genes contained in the PAM50 subtype predictor (BIRC5, CCNB1, CDC20, CDCA1, CEP55, KNTC2, MKI67, PTTG1, RRM2, TYMS and UBE2C) as surrogate signature biomarker of its anticancer activity.

VENTANA is a proof-of-concept study to describe the change in the expression of a proliferation-related gene signature in all 3 treatment arms. Changes in the proliferation signature will be determined by following formula: Mean suppression of proliferation signature score = $100 - [\text{geometric mean (post treatment proliferation score / pre-treatment proliferation score} \cdot 100)]$. By evaluating other BC-related gene signatures (560 genes), the antiangiogenic and immunogenic potential of the treatment arms will also be compared and genes regulated in a treatment-specific manner identified. All analyses will be performed within the different PAM50-defined subtypes (Luminal, Luminal A or Luminal B).

As the primary endpoint is continuous and there are no previous data to make assumptions about the degree of suppression of these genes, the sample size has not been determined by statistical calculations. A sample size of 20 pts per arm is considered appropriate to support our hypothesis. The targeted accrual of 60 pts will be enrolled in 10 sites across Spain (EudraCT Number 2015-004714-24).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-05

Title: PAINTER: Evaluation of eribulin tolerability and correlation between a set of polymorphisms and neuropathy in patients with metastatic breast cancer

La Verde N, Moretti A, Damia G, Paternò E, Santini D, Garrone O, Fabi A, Ciccarese M, Cretella E, Torri V, Generali D, Grasso D, Puglisi F, Collovà E, Roila F, Bertolini A, Barni S, Vici P, Luigi C, Scandurra G, Bramati A, Dazzani MC C and Farina G. ASST Fatebenefratelli Sacco, PO Fatebenefratelli, Milan, Italy; Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy; Campus Bio-Medico, University of Rome, Rome, Italy; Unit of Medical Oncology, S. Croce & Carle Teaching Hospital, Cuneo, Italy; Medical Oncology A, INT Regina Elena, Rome, Italy; Vito Fazi Hospital, Lecce, Italy; Medical Oncology, Azienda Sanitaria dell'Alto Adige, Bolzano, Italy; Laboratory of Methodology for Biomedical Research, IRCCS-Mario Negri Institute for Pharmacological Research, Milan, Italy; Unit of Molecular Therapy & Pharmacogenomic, AO Azienda Istituti Ospitalieri di Cremona, Cremona, Italy; Medical Oncology, IRCCS Foundation, San Matteo Hospital, Pavia, Italy; University Hospital of Udine, Udine, Italy; Oncology Unit, AO Ospedale Civile di Legnano, Legnano, Italy; Medical Oncology, Santa Maria Hospital, Terni, Italy; Medical Oncology Unit, A.O. Valtellina e Valchiavenna, Sondrio, Italy; Treviglio-Caravaggio Hospital, Treviglio, Italy; "Regina Elena" National Cancer Institute, Rome, Italy; Ospedale Guglielmo da Saliceto, Piacenza, Italy and Cannizzaro Hospital, Catania, Italy.

Body: BACKGROUND Eribulin is a synthetic analogue of halichondrin B which inhibits microtubule dynamics. It has been approved in Europe for the treatment of locally advanced or metastatic breast cancer (mBC) progressed after at least two chemotherapy regimens for advanced disease. The most common adverse events (AEs) were fatigue, neutropenia and peripheral neuropathy, which occurs with an incidence ranging from 13.9% to 35%. It was severe only in a small proportion of patients, suggesting an individual susceptibility. The neurotoxicity mechanisms associated with microtubulin interfering agents have not been fully defined. Few studies reported an association between some SNPs (Single Nucleotide Polymorphisms) and microtubulin interfering agents-induced neuropathy, mainly taxanes. As the use of Eribulin becomes more widespread, a better knowledge of its safety profile, outside of clinical trials, is warranted. Given that Eribulin toxicity can result in treatment discontinuation, the ability to anticipate which patients will experience severe toxicity could allow for either early intervention or even possibly for prophylactic therapy, or for a better selection of patients eligible for treatment.

METHODS This is a multicenter, interventional, single-arm, phase IV study. The primary objective is the evaluation of the safety and tolerability profile of Eribulin in an unselected population of patients with mBC. Secondary objectives are the description of compliance to treatment and efficacy.

ENDPOINTS

- Incidence, time of onset, severity and duration of all AEs experienced during treatment with Eribulin, especially the most common reported in previous studies but also other possible unexpected toxicities.
- Association between a set of selected SNPs and the onset of any grade peripheral neuropathy. Specifically, 15 SNPs located in genes involved in microtubule dynamics or resulted from genome wide association studies, will be analyzed.
- Evaluation of quality of life during treatment using validated questionnaires.
- Assessment of dose intensity and dose schedule maintenance.
- DOT (Duration Of Treatment) and OS (Overall Survival).

STATISTICAL METHODS Summary statistics will be used in order to describe patient characteristics. Safety endpoints will be estimated by means of absolute and relative frequencies and associated 95%CI. The relationship between baseline variables and the risk of severe toxicity, as well as the relationship between SNPs and risk of neuropathy will be described by means of contingency tables and their association with outcome will be assessed by χ^2 test of Mantel-Haenzel and a logistic regression model. DOT and OS will be described using Kaplan-Meier curves. A sample size of 200 patients will also allow us to get a good fitting for statistical analysis of the relationship between primary endpoint and not more than 10 factors. Regarding the relationship between SNPs and risk of neuropathy it will be feasible to screen for association about 10-15 SNPs, with known prevalence >15%.

PRESENT ACCRUAL AND TARGET ACCRUAL 98 of 200 patients were enrolled until 18/05/2016. Target accrual is open for 200 patients.

CONTACT INFORMATION Nicla La Verde: nicla.laverde@asst-fbf-sacco.it.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-06

Title: PIQHASSO: Open label, non-randomized, multicenter phase 1/2b study investigating safety and efficacy of PQR309 and eribulin combination in patients (pts) with locally advanced (LA) or metastatic HER2 (-) and triple-negative breast cancer (TNBC) (study PQR309-007)

López-Miranda E, Gávila J, Pernas S, Saura C, Oliveira M, Serra V, Schmid P, Lord S, Paez D, Perez J, Llombart A, Petrovic K, Dimitrijevic S and Cortes J. Hospital Ramón y Cajal, Madrid, Spain; IVO, Valencia, Spain; ICO L'Hospitalet, Hospitalet de Llobregat, Barcelona, Spain; Hospital Vall Hebrón, Barcelona, Spain; Barts Cancer Institute, London, United Kingdom; Churchill Hospital, Oxford, Headington, United Kingdom; Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; Baselga Institute of Oncology - Quiron, Barcelona, Spain; Hospital Arnau de Vilanova, Valencia, Spain; Medica Scientia Innovation-MedSIR ARO, Barcelona, Spain and PIQUR Therapeutics AG, Basel, Basel, Switzerland.

Body: BACKGROUND:

The PI3K/AKT/mTOR (PAT) pathway alteration has been strongly implicated in breast cancer and may contribute to resistance to available therapy. PQR309 is an oral pan-PI3K and mTOR inhibitor that penetrates the blood-brain barrier. Experiments of eribulin in combination with PI3K inhibitors in luminal and TNBC pre-clinical models enhanced antitumor activity.

TRIAL OBJECTIVES:

The primary objectives of the study are: to identify the maximum tolerated dose (MTD), evaluate the efficacy, safety and tolerability as well as the pharmacokinetics (PK) of PQR309 in combination with eribulin. In addition, exploratory objectives include assessments of: PAT pathway alterations prior to treatment, pharmacodynamics (PD) activity of PQR309 in combination with eribulin and correlation of PAT pathway alterations and PD activity with PQR309 and eribulin PK.

TRIAL DESIGN:

This is an open label, non-randomized, multicenter phase 1/2b clinical trial (dose escalation followed by expansion part) of PQR309 p.o. in combination with the standard dose of eribulin mesylate (1.4 mg/m²) in patients with LAMBC until progression or unacceptable adverse events (AE).

The dose escalation part of the study will first investigate PQR309 administered in a continuous daily (q.d.) and two intermittent treatment schedules in combination with standard administration of eribulin mesylate in patients with HER2 negative LAMBC following the "modified" 3 by 3 design. MTD is defined as the highest dose level at which ≤ 1 of 6 pts experiences dose-limiting toxicity (DLT) during the 1st cycle. After the MTD of PQR309 in combination with eribulin has been defined in all the three treatment schedules, one schedule will be selected, based on the overall evaluation of clinical data from the dose escalation part of the study, for further evaluation of efficacy in the expansion part of the study.

The expansion part of the trial applies Simon's MiniMax two-stage design. At the first stage, > 3 pts with TNBC with clinical benefit (CB) among 14 pts will be necessary to continue to the second stage. At the study end, > 9 pts with CB out of 28 pts are required to reject the null hypothesis. With this design, there is an 80% probability of a positive finding if the true clinical benefit rate (CBR) is $\geq 43\%$ and a 5% probability of a positive finding if the true CBR is $\leq 21\%$.

ELIGIBILITY:

Women with HER2- LAMBC with two to 5 prior chemotherapy regimens in advanced disease. Adequate organ function and performance status. Phase II specific selection criteria are: triple negative LAMBC and RECIST v1.1 evaluable disease.

ACCRUAL:

Approximately 60 patients will be enrolled in approximately 10 sites

Recruitment opened in March 2016.

TRIAL REGISTRATION: NCT02723877. Date of registration: 21/12/2015. First patient included: 04/04/2016.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-07

Title: A phase 2 study of eribulin followed by doxorubicin and cyclophosphamide as preoperative therapy for HER2-negative inflammatory breast cancer

Overmoyer B, Goel S, Regan M, Hirshfield-Bartek J, Schlosnagel E, Yeh E, Qin L, Bellon J, Nakhliis F, Jacene H and Winer E. Dana Farber Cancer Institute, Boston, MA and Brigham and Women's Hospital, Boston, MA.

Body: Background: HER2 negative(neg) inflammatory breast cancer(IBC) exhibits relative resistance to chemotherapy evidenced by pCR(pathologic complete response rate) rates of 12-25% with preoperative taxane/anthracycline regimens. Eribulin(Eisai®) inhibits microtubular function via sequestration of tubulin into nonfunctional aggregates, thus being effective against taxane-resistant cancer. Preclinical data shows 2 mechanisms of action: reversion of EMT(epithelial to mesenchymal transition) and normalization of tumor vascularity. Treatment of triple negative breast cancer(TNBC) cell-lines with eribulin results in downregulation of mesenchymal markers with concomitant increase in expression of classical epithelial markers(Yoshida BJC 2014). In PDX models, eribulin improved blood perfusion in central region of tumors, increased vessel density, reduced vessel diameter, and reduced hypoxia. IBC is highly angiogenic, with increased microvessel density, higher fraction of proliferating endothelial cells and greater expression of pro-angiogenic genes compared with non-IBC(McCarthy CCR 2002). This preoperative study(EAC) exploits the angiogenic properties of IBC with the treatment scheme of eribulin(E) followed by doxorubicin/cyclophosphamide(AC) in newly diagnosed HER2neg IBC.

Methods: Pts with HER2neg Stage III (cT4d,any N,M0) IBC are eligible if they have not received prior therapy for BC, have adequate organ function, cardiac ejection fraction $\geq 50\%$, and willing to undergo 2 research biopsies (rbx) of the affected breast. Following baseline rbx, pts receive cycle 1, day(d)1 eribulin 1.4 mg/m². A 2nd rbx occurs on d8, prior to dosing of E. Following 3 more cycles of E(1.4mg/m² d1,d8, every 21d), pts receive 4 cycles of dose-dense AC(A-60 mg/m²,C-600mg/m² every 14d). Pts with adequate disease response undergo mastectomy/axillary lymph node dissection followed by chest wall/regional lymph node radiation. Adjuvant endocrine therapy is used if hormone receptor positive. An imaging sub-study evaluates tumor perfusion via DCE-MRI pre and post 1st dose E.

Correlatives: To investigate whether E induces reversion of EMT in IBC, expression of 10 EMT-related genes are determined in each rbx, and normalization of tumor vessel phenotype are assessed by expression of 15 angiogenesis-related genes in rbx by RT-qPCR. Gene expression will be repeated on residual tumor at mastectomy. An imaging sub-study of DCE-MRI (10 pts) will assess vascular remodeling via changes in K^{trans} , v_e and v_p determination of IBC region of interest, core and rim and changes in the iAUC computed pre and post 1st dose E.

Statistics: The primary endpoint is pCR. A Simon two-stage design is used. If the proportion of pts having pCR is ≤ 0.10 then EAC is considered minimally effective, versus alternative hypothesis that EAC is worthy of further study if proportion pCR ≥ 0.30 . In the 1st stage, if $\leq 2/16$ pts have pCR, the study is stopped; if ≥ 3 pts have pCR, the study proceeds. In the 2nd stage, EAC is rejected if ≤ 4 of 25 pts have a pCR($\alpha=0.10$; $\beta=0.10$). Up to 25 pts will be enrolled. Secondary endpoints are residual cancer burden, disease-free survival, time to treatment failure and overall survival. Clinical trial information: [NCT02623972](https://clinicaltrials.gov/ct2/show/study/NCT02623972).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-08

Title: Intensified alkylating chemotherapy in patients with oligo-metastatic breast cancer harboring homologous recombination deficiency: The OLIGO study

Steenbruggen TG G, Vrancken Peeters M-JTFD TFD, Scholten AN N, Schot M, Wesseling J, Linn SC C and Sonke GS S.
Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands.

Body: Background Approximately 5% of patients with metastatic breast cancer survive more than 10 years. Long-term survival is mostly seen in patients with limited metastatic disease, often referred to as 'oligo'-metastatic disease. Oligo-metastatic breast cancer is variably defined as a maximum of 3-5 metastases beyond the regional lymph nodes. Some believe that oligo-metastatic cancer can be treated with curative intent using a multidisciplinary approach that targets the detected metastases, circulating micro-metastases, and any locoregional disease if present. Optimal patient selection is of vital importance.

Intensified alkylating chemotherapy in the treatment of breast cancer patients is controversial, as older studies have not shown a survival benefit in unselected groups of patients. More recent retrospective analyses, however, have suggested that patients with homologous recombination deficiency (HRD) derive significant benefit from intensified chemotherapy in comparison to conventional chemotherapy.

Trial design In this phase 3 trial patients with oligo-metastatic breast cancer and HRD start with 3 cycles of induction chemotherapy. Chemotherapy schedule includes anthracyclines and taxanes in treatment naïve patients and is personalized according to previously received (neo-)adjuvant chemotherapy in others. Patients with at least stable disease after 3 cycles are 1:1 randomized to receive another 3 cycles of conventional chemotherapy or progenitor cell mobilization with cyclophosphamide followed by 2 cycles of intensified chemotherapy (carboplatin 400 mg/m² (day 1&2), thiotepa 250 mg/m² (day 2), and cyclophosphamide 3000 mg/m² (day 1)) and peripheral blood progenitor cell reinfusion. Following systemic treatment, all patients receive maximal local therapy of locoregional and distant disease with surgery and/or radiotherapy.

Eligibility criteria Eligible patients have histologically proven, HER2 negative, oligo-metastatic breast cancer (1-3 distant metastatic lesions), with or without locoregional disease, either as *de novo* disease or recurrence. All lesions must be amenable to surgery or radiotherapy with curative intent. The tumor has to be deficient in homologous recombination by array comparative genomic hybridization and no prior chemotherapy for metastatic disease is allowed.

Specific aim To study the difference in event-free survival (EFS) between intensified alkylating chemotherapy compared to standard chemotherapy as part of a multimodality treatment approach in patients with oligo-metastatic breast cancer harboring HRD.

Statistical methods and patient accrual Primary endpoint of the study is EFS at 3 years. Toxicity, time to recurrence, and overall survival will be evaluated as secondary endpoints. A total of 65 EFS events will provide 80% power to detect a hazard ratio of 2.0 between treatment arms at the 0.05 two-sided significance level. Assuming an accrual period of 48 months and a maximum follow-up time of 60 months, 86 patients are required. At the time of abstract submission, 33 patients were randomized.

Contact information Principal investigator: Dr. GS Sonke, g.sonke@nki.nl. Study coordinator: TG Steenbruggen, t.steenbruggen@nki.nl. Clinicaltrials.gov: NCT01646034.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-09

Title: Feasibility and safety of avoiding granulocyte colony-stimulating factor prophylaxis during the paclitaxel portion of dose dense doxorubicin-cyclophosphamide and paclitaxel regimen

Barroso-Sousa R, Vaz-Luis I, Guo H, Barry WT T, Brackett AM M, Brock VA A, Roche KA A, Kasparian E, Winer EP P and Lin NU U. Dana-Farber Cancer Institute, Boston, MA.

Body: Background: The need for granulocyte-colony stimulating factor (G-CSF) support during dose-dense (DD) paclitaxel (T) after doxorubicin and cyclophosphamide (AC) is unclear. Given that G-CSF is not devoid of adverse effects, and adds significant costs to treatment, we are examining the feasibility and safety of avoiding G-CSF during dose dense T. **Methods:** This is a single center, single-arm, phase II, two stage study. The primary aim is to evaluate the rate of T treatment completion within 7 weeks (from D1 of cycle 1 to D1 of cycle 4 of T) omitting Pegfilgrastim using pre-specified safety rules. Secondary aims include: characterization of the utilization of Pegfilgrastim using pre-specified safety rules in patients receiving dose dense T; evaluation of the safety of omitting routine Pegfilgrastim support in patients receiving dose dense T; evaluation of total cost (\$ United States) of omitting routine Pegfilgrastim use during dose dense T. As a secondary aim we will evaluate the safety of simplifying the pre-medication regimen used for the T portion of the regimen (withholding corticosteroids in cycle 3 and 4 if no evidence of allergic reactions in cycle 1 and 2). A Simon Optimal design was selected with an overall one-side type I error of 10% and 90% power to detect the difference between unacceptable T completion rate (75%) and desirable completion rate (85%). In the first stage, 51 evaluable patients will be enrolled. If during the first stage, at any point, a total of 12 or more patients do not complete treatment within 7 weeks the trial will be closed permanently. Among the 51 patients enrolled after the first stage, if at least 40 patients complete treatment without dose delay, accrual will continue to the second stage where an additional 74 evaluable patients will be enrolled. If there are at least 100 among the 125 evaluable patients completing treatment without dose delay, the regimen will be considered worthy of further study. If during the second stage, at any point, a total of 26 patients do not complete treatment within 7 weeks the trial will be closed permanently and the study intervention will not be of clinical interest. If the true treatment completion rate is 75%, the chance the regimen is declared ineffective is 91% (exact alpha = 0.094) and if the true treatment completion rate is 85% the chance that the regimen is falsely declared ineffective is 10% (exact power = 0.899). The estimated accrual rate is 6-8 patients/month. Accrual started in April 2016. Clinical trial information: NCT02698891.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-10

Title: Multi-center phase IB trial of ACY-1215 (Ricolinostat) combined with nab-paclitaxel in unresectable or metastatic breast cancer

Onishi M, Chiuzan C, Fasano J, Crew K, Accordino M, Tiersten A, Shapiro C, Jarpe M, Qualye S, Trede N, Wheeler C, Hershman D, Silva J, Maurer M and Kalinsky K. Columbia University Medical Center, New York, NY; Mount Sinai Hospital, New York, NY and Acetylon Pharmaceuticals, Inc., Boston, MA.

Body: BACKGROUND:

HDAC6, a cytoplasmic histone deacetylase, targets non-chromatin substrates including tubulin, HSP90, and cortactin, playing an important role in cell-cell interactions, motility, chaperone function, and protein degradation. ACY-1215 (Ricolinostat) is an orally active, selective HDAC6 inhibitor. Preclinical studies have demonstrated ACY-1215 to have activity in breast cancer, synergistic cytotoxicity with taxanes, possibly due to combined effects on microtubule stabilization, and protective effects against neurodegeneration and potentially peripheral neuropathy. Analysis of human primary breast cancer gene expression data has identified a set of 162 genes corresponding to a HDAC6 master regulator (MR) score that correlates with IC50 or sensitivity to ACY-1215, and may serve as a potential biomarker.

TRIAL DESIGN:

This is an open-label, multicenter Phase 1b trial (NCT01323751) of ACY-1215 combined with nab-paclitaxel in unresectable or metastatic breast cancer. Patients will receive ACY-1215 daily for 21 days of each 28-day cycle in combination with nab-paclitaxel 100 mg/m² on days 1, 8, and 15 until progression of disease or unacceptable toxicity.

KEY ELIGIBILITY CRITERIA:

Patients aged ≥ 18 years with unresectable or metastatic breast adenocarcinoma, measurable or non-measurable disease, an ECOG performance status of 0 to 1, baseline toxicities and symptoms grade ≤ 1 , and at least 2 weeks since prior treatment.

STATISTICAL METHODS:

Dose escalations will be performed according to the time to event continual reassessment method (TITE-CRM), starting at 120 mg to a maximum dose of 240 mg. The maximum tolerated dose (MTD) will be identified as the dose level associated with a target probability of dose limiting toxicity (DLT) of 0.25. The TITE-CRM will use an empirical dose-toxicity model, with a sample size of 24. The dose-toxicity model is calibrated to select a dose that yields between 17 and 33% DLT, which will be the recommended phase II dose (RP2D).

OBJECTIVES:

The primary objective of this trial is to determine the MTD of ACY-1215 in combination with weekly nab-paclitaxel. Secondary objectives include the evaluation of safety and tolerability, progression free survival, overall response rate (measured by RECIST 1.1), and clinical benefit rate. Exploratory analyses will assess pharmacokinetics and pharmacodynamics, correlation of biomarkers including the HDAC6 score with response, and the effect of ACY-1215 on taxane-induced peripheral neuropathy.

ACCRUAL: Target Accrual is 24 patients.

CONTACT INFORMATION:

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2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-11

Title: Randomized phase 3 study of a novel, long-acting G-CSF (eflapegrastim) versus pegfilgrastim in the management of chemotherapy-induced neutropenia in early-stage breast cancer patients receiving docetaxel and cyclophosphamide (TC) (ADVANCE study)

Schwartzberg LS S, Bharadwaj J, Peguero JA A, Vacirca JL L, Ibrahim EN N, Bhat G, Choi MR, McGregor K and Agajanian R. West Cancer Center, Memphis, TN; Oncology Consultants, Houston, TX; North Shore Hematology/Oncology Associates, East Setauket, NY; Beaver Medical Group, Highland, CA; Spectrum Pharmaceuticals, Irvine, CA; Samaritan Hematology and Oncology Associates, Corvallis, OR; The Oncology Institute of Hope and Innovation, Downey, CA and Pacific Cancer Medical Center, Anaheim, CA.

Body: Background: Eflapegrastim is a distinct biologic that uses an innovative, proprietary long-acting protein/peptide discovery technology (LAPSCOVERY™). Eflapegrastim consists of a novel, modified recombinant human G-CSF conjugated to the Fc fragment of IgG4 via a polyethylene glycol linker to produce a new, longer-acting G-CSF with a potentially unique distribution to areas rich in Fc receptors including its site of action in the bone marrow. A successful dose-finding Phase 2 trial including a pegfilgrastim control arm established the dose for a Phase 3 non-inferiority trial.

Trial Design: This is a randomized, open-label, active-controlled, multinational, multicenter, Phase 3 study comparing the efficacy and safety of eflapegrastim to pegfilgrastim. Patients (n=580) will be randomized in a 1:1 ratio to receive either eflapegrastim (equivalent to 3.6 mg G-CSF) or pegfilgrastim (equivalent to 6.0 mg G-CSF) once per chemotherapy cycle (up to 4 cycles), approximately 24 hours after chemotherapy. The primary endpoint is to compare the efficacy of a single dose of eflapegrastim with pegfilgrastim in patients with ESBC receiving TC, as measured by the Duration of Severe Neutropenia (**DSN**) in Cycle 1. Key secondary objectives include Time to Absolute Neutrophil Count (ANC) Recovery in Cycle 1; Depth of ANC Nadir in Cycle 1; incidence of Febrile Neutropenia. Safety and pharmacokinetics will also be assessed.

Eligibility Criteria: This study is enrolling histologically confirmed ESBC patients who are: eligible to receive adjuvant or neoadjuvant TC chemotherapy; at least 18 years of age, with adequate hematologic, renal and hepatic function. Patients will be excluded if they have: active concurrent malignancy or life-threatening disease; a known sensitivity or previous reaction to E. coli derived products or any of the products to be administered during study participation; concurrent adjuvant cancer therapy; locally recurrent/metastatic or contralateral breast cancer; previous exposure to filgrastim, pegfilgrastim, or other G-CSF products in clinical development prior to the administration of study drug; bone marrow or hematopoietic stem cell transplant or radiation therapy prior to enrollment, or are pregnant or breast-feeding.

Statistical Methods: The goal of this study is to demonstrate non-inferiority. For the Primary Efficacy Analysis, the mean **DSN** in Cycle 1 will be compared between the eflapegrastim and pegfilgrastim treatment arms. A 2-sided 95% confidence interval (CI) of the difference between the mean **DSN** of the eflapegrastim arm and the mean **DSN** of the pegfilgrastim arm will be calculated using bootstrap resampling with treatment as the only stratification factor. For the Secondary Efficacy Analyses, the results will each be summarized by treatment arm and cycle. The two-sided 95% CI for the difference between the treatment arms will be calculated.

Target Accrual: Approximately 580 patients. Enrollment began January 2016.

Contact Information: Spectrum Pharmaceuticals. advance@sppirx.com.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-12

Title: A phase II, multicenter, randomized trial of eribulin plus gemcitabine (EG) vs. paclitaxel plus gemcitabine (PG) in patients with HER2-negative metastatic breast cancer as first-line chemotherapy

Park YH, Im S-A, Sohn JH, Lee KS, Chae YS, Lee KH, Kim J-H, Im Y-H, Ahn JS, Kim T-Y, Lee K-H, Kim S-B, Ahn J-H, Kim GM, Park IH, Lee SJ, Han HS, Kim SH and Jung KH. Samsung Medical Center; Seoul National University Hospital; Yonsei Cancer Center; National Cancer Center; Kyungpook National University Medical Center; Chungbuk National University Hospital; Seoul National University Bundang Hospital and Asan Medical Center.

Body: Background: Metastatic breast cancer (MBC) is an incurable disease and is needed to improve effective chemotherapy. Paclitaxel plus Gemcitabine (PG) combination chemotherapy is one of the preferred chemotherapeutic regimens for patients with MBC, and was found to be proper as a maintenance chemotherapy regimen with survival benefit and feasible toxicity profile. Eribulin mesylate is a non-taxane inhibitor of microtubule dynamics of the halichondrin class of antineoplastic drugs. A recent pooled analysis of two phase II studies with eribulin showed improved overall survival in various patient subgroups with advanced/metastatic breast cancer who had previously received an anthracycline and a taxane. Furthermore, eribulin may have rational benefit compared with paclitaxel in terms of neurotoxicity. Therefore, Eribulin plus Gemcitabine (EG) combination chemotherapy may have less neurotoxicity comparing to PG.

Trial Design: Prospective randomized phase 2, open-label, two-arm, multi-center study comparing EG chemotherapy with PG chemotherapy for patients with HER-2 negative MBC as first-line chemotherapy.

Eligibility Criteria: Histologically confirmed breast cancer patients, at least 19 years of age, with no prior history of chemotherapy for metastatic, recurrent breast cancer with evaluable lesions (as per RECIST, 1.1) who have adequate hematologic, renal, and hepatic function. Patients either may or may not have a prior anthracycline containing regimen. Prior hormonal therapy as a treatment of metastatic disease is allowed.

Specific Aims:

The primary efficacy endpoint of the trial is Progression-Free Survival (PFS). The secondary efficacy endpoints are: Time to Treatment Failure (TTF); Overall Survival (OS); neuropathic scale (FACT for Taxane QOL assessment); toxicity; duration of response; Objective Response Rate (ORR); Clinical Benefit Rate. The exploratory endpoint of the study includes pharmacogenetic profile.

Statistical Methods:

The initial sample size of the present study was determined based on the data derived from a previous trial on PG maintenance chemotherapy design; 6-month PFS is 70% for PG chemotherapy. This design was hypothesized that EG chemotherapy would not be inferior to PG chemotherapy. Thus, estimated PFS for each arm is 70%. Based on this estimate, we would plan to recruit a total of 100 patients (50 per arm). Considering drop-out rate of 10%, total 112 MBC patients planned to be enrolled.

Present Accrual and Target Accrual: Enrollment has been completed as of March, 2016 with a target enrollment of 112 patients.

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ClinicalTrials.gov Identifier: NCT02263495.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-01

Title: Phase II neoadjuvant trial of nanoparticle albumin-bound paclitaxel and trastuzumab in patients with node-negative, Her-2 positive breast cancer (OMC-BC04)

Iwamoto M, Tanaka S, Koda C, Kawaguchi K, Terasawa R, Sato N, Fujioka H, Kimura K and Uchiyama K. Osaka Medical College, Osaka, Japan and Osaka Minami Medical Center, Osaka, Japan.

Body: Background: Neoadjuvant chemotherapy plus trastuzumab results in a 30% to 50% pathologic complete response (pCR) rate in HER-2 positive breast cancer and has been associated with improved therapeutic outcomes. Thus, the pCR rate can be useful in evaluating novel agents in this patient population. Albumin-bound (nab)-paclitaxel can reduce the toxicity of Paclitaxel while maintaining its efficacy. We reported that neoadjuvant therapy using Anthracycline based regimens (EC,AC,FEC) followed by a combination with nab-Paclitaxel and Trastuzumab was effective and safe by OMC-BC01 Study (Tanaka et al. Clin Breast Cancer 15:191-196). The pCR rate was 36% and 71% in the patients with estrogen receptor-positive and negative cancer, respectively. In addition, Tolaney et al. showed that adjuvant Paclitaxel and Trastuzumab for node-negative, HER-2 positive tumors measuring up to 3 cm in greatest dimension was associated with patients outcomes that were better than expected on the basis of historical data (Tolaney et al. N Engl J MED.2015 Jan 8:372(2):134-141). We conducted a clinical Phase II, multicenter, neoadjuvant trial of combination with nanoparticle albumin-bound Paclitaxel and Trastuzumab in patients with node-negative, Her-2 positive, estrogen receptor-negative breast cancer measuring up to 3 cm in greatest dimension.

Patients and Methods: nab-Paclitaxel and Trastuzumab as neoadjuvant therapy in patients with Her-2 positive, node-negative, estrogen receptor-negative breast cancer measuring up to 3 cm in greatest dimension. Patients are treated with neoadjuvant nab-Paclitaxel (260mg/m²) and Trastuzumab q21d x 4, and undergo surgery 4-6 weeks later from completing chemotherapy. The primary endpoint, pCR is defined as no evidence of invasive tumors in the final surgical sample both in the breast and axillary lymph nodes. Secondary endpoints include objective clinical response rate, histological response rate, disease-free interval, rate of breast conserving surgery, and the safety of the treatment.

Accrual: Presently, a total number of 1 patient have been included since start of the study. The expected end of accrual of 30 patients will be the last quarter 2018.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-02

Title: Prospective evaluation of the predictive value of biomarkers with complete pathologic response using neoadjuvant chemotherapy plus trastuzumab + pertuzumab in Her2 (+) breast cancer patients with invasive tumors

Diaz-Cantón E, Jankilevich G, Denninghoff V, Elizalde P, Galanternik F, Bianconi MI, Recondo G and Storino C. Instituto Universitario CEMIC (IUC), Buenos Aires, Buenos Aires (Ciudad), Argentina; Hospital Carlos G Durand, Buenos Aires, Buenos Aires (Ciudad), Argentina and Instituto de Biología y Medicina Experimental (IBYME), Buenos Aires, Buenos Aires (Ciudad), Argentina.

Body: Preoperative treatment with anthracycline/taxane containing chemotherapy plus trastuzumab and pertuzumab (CT/T/P) is a standard treatment in patients with Her 2 (+) invasive breast cancer requiring systemic therapy.

Patologic complete response (pCR) is strongly associated with disease free survival (DFS) in Her2 (+) tumors.

We want to correlate immune, inflammatory, and genetic characteristics before and after neoadjuvant CT/T/P in the cohort of patients depicted above.

We plan to include 40 patients in 2 years in this pilot, prospective, observational and multicentric study.

After signature of an informed consent a core biopsy is going to be undertaken.

Before given any treatment, we are going to evaluate: stromal tumor infiltrating lymphocytes (sTILs), CD8, PD1, PDL1, the mutational status of PI3K and the status of the STAT 3 pathway.

CT/T/P x 6 cycles is going to be administered followed by the corresponding surgery.

Afterwards, the same biomarkers are planned to be measured in the surgical specimen.

Correlation of each one of the biomarkers with pCR will be evaluated. Discrete variables will be evaluated by logistic regression, Categorical variables are planned to be studied with uni and multivariate analysis.

Upon completion of the enrollment of the patients the analysis of DFS and overall survival will be pursued.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-03

Title: Phase I multicenter clinical trial evaluating the combination of trastuzumab emtansine (T-DM1) and non-pegylated liposomal doxorubicin (NPLD) in HER2-positive metastatic breast cancer (MBC) (MEDOPP038 study)

López-Miranda E, Brain E, Saura C, Gligorov J, Dubot C, Dieras V, Suter TM M, Aguirre E, Perez-García JM, Llombart A and Cortés J. Ramón y Cajal University Hospital, Madrid, Spain; Institut Curie / Hôpital René Huguenin, St Cloud, France; Vall d'Hebrón University Hospital, Vall d'Hebrón Institute of Oncology (VHIO), Barcelona, Spain; APHP Tenon, IUC-UPMC, Sorbonne University, Paris, France; Institut Curie, Paris, France; Bern University Hospital, Cardiology, Bern, Switzerland; Medica Scientia Innovation Research – MedSIR ARO, Barcelona, Spain; Baselga Institute of Oncology, Quiron University Hospital, Barcelona, Spain and Hospital Arnau i Vilanova, Valencia, Spain.

Body: BACKGROUND:

Clinical efficacy and safety of T-DM1 for the treatment of HER2-positive MBC has been assessed in several phase II and III trials and is now considered the standard of care in taxane-and trastuzumab-progressing patients. However, although T-DM1 has shown encouraging antitumor activity in the advanced setting, several strategies to improve T-DM1 efficacy are currently evaluated, including the combination with non-pegylated liposomal doxorubicin (NPLD), considering that: i) doxorubicin is one of the most active chemotherapeutic agents against HER2-positive breast cancer; ii) the combination of doxorubicin and trastuzumab induces synergistic antitumor activity in HER2-overexpressing preclinical models; and iii) liposomal formulations of doxorubicin *have a reduced* risk of developing cardiac toxicity.

OBJECTIVES:

The primary objective of this trial is to determine the maximum tolerated dose (MTD) of the combination of T-DM1 and NPLD in patients with HER2-positive MBC naïve of anthracyclines and previously treated with trastuzumab and a taxane. The secondary objectives include 1) safety, with special emphasis on cardiac safety evaluated by left ventricular ejection fraction, high-sensitivity troponin I and B-type natriuretic peptide (BNP) levels, 2) pharmacokinetics, 3) antitumor activity, and the 4) role of single nucleotide polymorphisms of *HER2* gene for developing cardiotoxicity.

TRIAL DESIGN:

This is a dose-finding, open-label, non-randomized and multicenter phase I clinical trial of T-DM1 at a fixed dose of 3.6 mg/kg IV in combination with three different dose levels (DL) of NPLD (45, 50, and 60 mg/m²) IV administered on Day 1 every three weeks. The trial follows a *modified* dose escalation scheme with a *3+3 design*. A total of three patients will be included in the first cohort and observed for dose-limiting toxicities (DLTs) during the first two cycles of treatment. If none of these patients experiences a DLT, three other patients will be treated at the next DL. However, in case of at least one patient experiences a DLT, three more patients will be treated at the same DL. The MTD will be defined as the highest DL at which ≤ 1 of six patients experiences a DLT during the first two cycles of treatment. An expansion cohort of six additional patients at the MTD will be included.

ELIGIBILITY:

Anthracycline-naïve patients with HER2-positive MBC and up to two prior chemotherapy regimens in the advanced setting who previously were treated with trastuzumab and a taxane. ECOG performance status of 0-1. Adequate organ and cardiovascular function with LVEF $\geq 55\%$. RECIST v1.1 evaluable disease.

ACCRUAL:

A total of 12-24 patients will be enrolled at four sites in Spain and France. Recruitment was opened on September 2015. To date, four patients (three at DL1 and one at DL2) have been recruited.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-04

Title: The DETECT V-study – Comparison of dual HER2-targeted therapy with trastuzumab plus pertuzumab in combination with chemo- or endocrine therapy in patients with HER2-positive and hormone-receptor positive metastatic breast cancer

Polasik A, Schramm A, Friedl TWP WP, Rack B, Trapp E, Tzschaschel M, Fasching PA A, Taran F-A, Hartkopf A, Schneeweiss A, Müller V, Aktas B, Pantel K, Meier-Stiegen F, Wimberger P, Janni W and Fehm T. Gynecology and Obstetrics, University Hospital Ulm, Ulm, Germany; Gynecology and Obstetrics, Klinikum der Ludwig-Maximilians-Universität, Munich, Germany; Gynecology and Obstetrics, University Hospital Erlangen, Erlangen, Germany; Gynecology and Obstetrics, University Hospital Tübingen, Tübingen, Germany; University Hospital Heidelberg, Germany; Gynecology and Obstetrics, University Hospital Hamburg-Eppendorf, Hamburg, Germany; Gynecology and Obstetrics, University Hospital Essen; University Hospital Hamburg-Eppendorf, Hamburg, Germany; Gynecology and Obstetrics, Heinrich-Heine-Universität; Düsseldorf, Germany and Gynecology and Obstetrics, University Hospital Dresden, Technische Universität Dresden, Germany.

Body: Background: Maintenance of quality of life (QoL) is one of the main aims of treatment of incurable diseases such as metastatic breast cancer (MBC). In patients with HER2-positive MBC, taxan-based chemotherapy in combination with dual HER2 targeted therapy with trastuzumab and pertuzumab has shown promising efficacy results in terms of prolonged survival. However, cytostatic treatment is often accompanied by adverse events of grade 3 or higher, seriously impacting the patients' QoL. In patients with HER2-positive and hormone-receptor positive MBC, the combination of trastuzumab with aromatase inhibitors was shown to be a safe and effective treatment option. The synergistic combination of dual HER2-targeted therapy with trastuzumab and pertuzumab plus endocrine therapy might offer an even better treatment option for these patients. DETECT V is the first prospective randomized phase III clinical trial comparing the safety and efficacy of the dual HER2-targeted therapy in combination with either endocrine therapy or chemotherapy.

Trial design and eligibility criteria: Women with HER2-positive and hormone-receptor positive MBC with first to third line therapy are 1:1 randomized either to a dual HER2-targeted therapy with Pertuzumab and Trastuzumab plus endocrine therapy or to the dual HER2-targeted therapy plus chemotherapy.

Specific aims: The primary objective of this study is to compare the safety and QoL in both arms, as assessed by the occurrence of AEs during the treatment period. We developed a modified adverse event score - including all adverse events grade 3 or higher, except neutropenia, which is included only if rated grade 4, and alopecia, rash, hand-foot-syndrome and peripheral neuropathy which are included if rated grade 2 or higher – in order to better reflect the clinical, physiological and psychological impact of AEs on patients' QoL. Key secondary endpoint, besides the efficacy endpoints progression free survival (PFS) and overall survival, is to compare quality-adjusted survival (QAS), as measured using the quality-adjusted time without symptoms and toxicity (Q-TWiST) method, between both treatment arms. QAS as measured using the Q-TWiST method provides a single metric value that is a composite measure of quantity of survival time and quality of survival as assessed by the patients themselves. Q-TWiST analyses account for possible trade-offs between quantity and quality of life (e.g. prolonged time to progression at the cost of higher toxicity, which adversely affects QoL), and provide an excellent tool to evaluate whether two treatment options differ with regard to the overall perceived value to the patients.

Translational research projects focus on Circulating Tumor Cell(CTC)-enumeration (the presence of CTCs is not obligatory in DETECT V), prognostic role of CTC dynamics, and the assessment of marker expression on CTCs in order to calculate an endocrine responsiveness score which will be evaluated regarding its suitability to predict treatment success.

Contact: For further information on the DETECT V study please contact www.detect-studien.de or studienzentrale.ufk@uniklinik-ulm.de.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-05

Title: Phase II clinical trial of neratinib in patients 60 and older with HER2 over-expressed or mutated breast cancer: Trial design considerations for older adults

Yuan Y, Blanchard S, Li D, Mortimer J, Waisman J, Somlo G, Yost S, Katheria V and Hurria A. City of Hope.

Body: Background: This study addresses a key knowledge gap identified by the Institute of Medicine report on quality cancer care. Although there has been a growth in the number of targeted agents approved for the treatment of breast cancer, there are limited data regarding the efficacy, toxicity, and management of side effects in older adults. Neratinib is a potent oral small molecule tyrosine kinase inhibitor. Early clinical data have demonstrated the activity of neratinib in patients who have already progressed through HER2 targeted therapies. This study is designed to evaluate the tolerability and toxicity profile of neratinib in older adults with metastatic breast cancer (MBC) incorporating geriatric oncology design considerations.

Trial Design: This is an open label, single arm, phase II study of single agent neratinib in patients with HER2 positive MBC. Neratinib is given at 240mg orally in 28 day cycles. Unique factors of this geriatric oncology trial design include: 1) pre-treatment and on-treatment geriatric assessment; 2) additional nurse toxicity visits; 3) an algorithm for aggressive management of diarrhea; 4) measurements of the pharmacokinetics (PK) of neratinib; 5) inclusion of biomarkers of aging; 6) measurement of patient adherence; and 7) evaluation of quality of life.

Eligibility Criteria: Patients must be age ≥ 60 with histologically-proven HER2 positive MBC or MBC with HER2 receptor activating mutations. There is no limitation on the number of previous lines of therapy, but patients must have adequate organ and bone marrow functions, and a baseline LVEF $\geq 50\%$. Exclusion Criteria include: prior treatment with neratinib; major surgery within 28 days; uncontrolled cardiac disease; concurrent use of digoxin; or chronic diarrhea.

Specific Aims: The primary objective of this study is to identify the rate of grade 2 or higher toxicities attributed to neratinib in adult age ≥ 60 with HER2 over-expressing breast cancer. The secondary objectives are to describe the full toxicity profile (including all grades of gastrointestinal toxicities); to estimate the rate of dose reduction, holds and hospitalizations; to describe the PK parameters; to estimate the adherence rate to neratinib; and to estimate the overall response, clinical benefit rate, progression-free and overall survival. Furthermore, we will explore the role of a cancer-specific geriatric assessment and serum biomarkers of aging (IL-6, CRP, and D-dimer) in predicting treatment toxicities and PK parameters.

Statistical Design: We plan to enroll 40 patients age ≥ 60 (at least 5 patients age 75 years or older, and no more than 15 patients 60-70) in order to assure that our sample is representative of the entire age range of older adults. Given a sample size of 40 subjects, the widest half-width of the 95% confidence limits for the rate of grade 2 or higher toxicities will be less than or equal to 0.16. An interim analysis will be performed after 20 subjects have been on study for at least one cycle.

Accrual goal: 40

Contact information: Yuan Yuan MD PhD, Email: yuyuan@coh.org.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-06

Title: HERMIONE: A phase 2, randomized, open label trial comparing MM-302 plus trastuzumab with chemotherapy of physician's choice plus trastuzumab, in anthracycline naïve HER2-positive, locally advanced/metastatic breast cancer patients previously treated with pertuzumab and ado-trastuzumab emtansine (T-DM1)

Miller K, Cortes J, Hurvitz SA A, Krop IE E, Tripathy D, Verma S, Dionne M, Reynolds JG G, Wickham T, Molnar I and Yardley DA A. Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN; Vall d'Hebron Institute of Oncology (VHIO) and Ramon y Cajal University Hospital, Barcelona and Madrid, Spain; University of California Los Angeles, Los Angeles, CA; Dana-Farber Cancer Institute, Boston, MA; MD Anderson Cancer Center; Sunnybrook Odette Cancer Centre, Toronto, Canada; Merrimack Pharmaceuticals, Inc., Cambridge, MA and Sarah Cannon Research Institute, and Tennessee Oncology, PLLC, Nashville, TN.

Body: Background: Although HER2-targeted therapies such as pertuzumab and T-DM1 have improved patient outcomes for HER2-positive metastatic breast cancer (MBC), treatment resistance typically occurs. MM-302 is a HER2-targeted liposomal doxorubicin in development by Merrimack Pharmaceuticals. In a Phase 1 study, patients with HER2-positive MBC were treated with MM-302 alone and in combination with trastuzumab with or without cyclophosphamide. MM-302 had an acceptable safety profile, and promising efficacy was observed in patients not previously exposed to an anthracycline.

Trial design: HERMIONE (NCT02213744) is a randomized Phase 2, two-arm, open-label trial designed to evaluate if MM-302 can address an unmet medical need in patients with anthracycline naïve, trastuzumab-, pertuzumab- and T-DM1-pretreated HER2-positive locally advanced breast cancer (LABC)/MBC. Patients are randomized 1:1 to receive MM-302 (30mg/m², Q3W) plus trastuzumab (6mg/kg, Q3W) or chemotherapy of physician's choice (vinorelbine, capecitabine, or gemcitabine) plus trastuzumab (6mg/kg, Q3W).

Eligibility criteria: Centrally confirmed HER2-positive LABC/MBC, no prior anthracycline exposure, prior trastuzumab in any setting, prior T-DM1 in the LABC/MBC setting, prior pertuzumab in LABC/MBC setting or disease recurrence within 12 months of neoadjuvant/adjuvant treatment, unlimited prior lines of therapy, ECOG 0-1 and LVEF ≥50%. CNS metastases are permitted if stable and without symptoms or steroids for 4 weeks.

Specific aims: The primary endpoint is progression free survival (PFS) as assessed by an independent blinded review of tumor assessments. Secondary endpoints include investigator assessed PFS, overall survival, response rate, safety and patient related outcomes.

Statistics: 250 patients will be enrolled to observe 191 PFS events for 90% power to detect a Hazard Ratio of 0.625. The MM-302 arm will be compared to the control arm on the primary endpoint of PFS using a stratified log-rank test at one-sided 0.025 level.

Accrual status: First patient treated was in December 2014 and enrollment is expected to be complete in 2017. Sites are open in the US, Canada and Western Europe and are currently enrolling patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-07

Title: SOPHIA: A phase 3, randomized study of margetuximab plus chemotherapy vs trastuzumab plus chemotherapy in the treatment of patients with HER2+ metastatic breast cancer

Rugo HS S, Pegram MD D, Gradishar WJ J, Cortes J, Curigliano G, Hong S, Wigginton JM M, Lechleider RJ J and Cardoso F. University of California, San Francisco, San Francisco, CA; Stanford School of Medicine, Stanford, CA; Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL; Vall d'Hebron Institute of Oncology, Barcelona, Spain; Istituto Europeo di Oncologia, Milano, Italy; MacroGenics, Inc., Rockville, MD and Champalimaud Cancer Centre, Lisbon, Portugal.

Body: Background: Despite significant advances in targeted therapy, HER2+ metastatic breast cancer (MBC) remains incurable. Ideal treatment includes pertuzumab and trastuzumab in combination with a taxane in the first line setting, followed by ado-trastuzumab emtansine on progression. Optimal treatment regimens in the third and greater line of therapy are not defined, but continued anti-HER2 therapy is recommended. Margetuximab is a Fc-modified monoclonal antibody to HER2 that recognizes the same epitope on HER2 as does trastuzumab, with similar affinity. Margetuximab demonstrates increased affinity to the activating CD16A Fc-receptor found on NK cells and macrophages and decreased affinity to the inhibitory CD32B receptor compared to trastuzumab. In vitro studies showed enhanced antibody dependent cell-mediated cytotoxicity compared to trastuzumab. In a Phase 1 dose escalation and expansion trial, margetuximab showed single agent clinical activity against HER2+ tumors in patients previously treated with trastuzumab and other anti-HER2 agents. Methods: SOPHIA is a randomized, prospective study testing the hypothesis that margetuximab plus chemotherapy (CTX) is more effective than trastuzumab plus CTX in patients previously treated for HER2+ MBC. Sequential primary endpoints are centrally assessed progression free survival (PFS) and overall survival (OS). The study size of 530 patients is determined to have 80% power to detect a hazard ratio for OS of 0.75. Secondary endpoints are investigator assessed PFS and centrally assessed overall response rate. Eligibility includes prior treatment with trastuzumab, pertuzumab, and ado-trastuzumab emtansine; no more than 3 prior lines of therapy in the metastatic setting; prior demonstration of HER2+ status at a local reference laboratory; and absence of active brain metastases. Eligible patients are randomized 1:1 to receive CTX (physician's choice: capecitabine, eribulin, gemcitabine or vinorelbine) plus either margetuximab or trastuzumab until disease progression or toxicity. Antibody may be continued after stopping CTX in patients with responding or stable disease. **Progress to date:** The trial was initiated July 2015 and is ongoing in the US and Europe with planned expansion to Korea and Israel. ClinicalTrials.gov Identifier NCT02492711; Eudract 2015-000380-13.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-08

Title: A phase II single arm trial to assess the efficacy of ASLAN001 plus capecitabine in previously irradiated, progressing central nervous system (CNS) metastases for HER2+ breast cancer patients

Lee GE. National Cancer Centre Singapore, Singapore.

Body: Brain metastasis in breast cancer (BMBC) has always been associated with very poor prognosis with no current standard of treatment. Treatment options generally involve local control. Subsequent to local treatment, systemic control can be re-initiated via systemic therapies or trial participation. ASLAN001 has been shown to be active in pre-clinical tumor models. It is a potent, specific, adenosine triphosphate competitive inhibitor of the tyrosine kinases of the cell surface receptors (HER)-1, HER-2, and HER-4. It also has a pre-clinical safety profile consistent with its known pharmacology. A Phase 1 study using ASLAN001 in combination with capecitabine showed a response rate of 38% in 20 HER2+ breast cancer patients.

This is a single arm phase II study. 29 patients with previously irradiated, progressing brain metastases in Her2 positive breast cancer will be enrolled to receive ASLAN001 (400mg bd daily) and capecitabine (1000mg/m² bd per day for day 1-14 of 21 day cycle). Treatment will continue until disease progression or unacceptable toxicity. Baseline brain imaging using either Magnetic resonance imaging (MRI) will be performed, with follow-up scans repeated every 2 cycles until progression. Non-brain imaging will also be performed at the same settings.

Specific Aims:

Evaluate the efficacy of ASLAN001 in combination with capecitabine in terms of CNS response

Evaluate the activity (clinical benefit) of ASLAN001 in combination with capecitabine in systemic disease (outside CNS)

Evaluate the tolerability of ASLAN001 in combination with capecitabine

Eligibility:

HER2 positive breast cancer patients with previously irradiated, progressing brain metastases.

Previously irradiated CNS metastases (WBRT or SRS or both).

Adequate end organ function.

No prior exposure to either lapatinib or capecitabine

Statistical Design:

In order to minimize the expected number of patients treated in the event that the regimen proves to be very disappointing or very successful; a two-stage design will be used for patient accrual (Simon 1989).

Stage 1 of accrual: 10 response evaluable patients will be entered in the first stage. Using response hypothesis of $H_0 < 5\%$ and $H_a > 20\%$, we would reject the drug combination at the end of the first stage of accrual if less than 1 response was observed.

Stage 2 of accrual: Additional 19 patients will be accrued. We would accept the drug combination as active if 4 or more objective responses are observed from a total of 29 patients accrued.

Significance level and power: To test the null hypothesis that the response rate is $< 5\%$ and rejects this in favour of the alternative hypothesis of response rate is $> 20\%$. The significance level (i.e., the probability of rejecting H_0 when it is true) is $\alpha = 5\%$ (1-tailed) and the power (i.e., 1-beta, the probability of deciding the regimen is active) is 80%. The expected sample size with this design is 29 using the optimal design criterion of Simon's two-stage method. At stage 1 (10 patients), at least 1 response needs to be seen in order to initiate stage two with the remainder of 19 patients. The trial will be positive if at the end 4 responses will be seen in the 29 patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-09

Title: A phase 2 randomized, double-blinded, controlled study of ONT-380 vs. placebo in combination with capecitabine (C) and trastuzumab (T) in patients with pretreated HER2+ unresectable locally advanced or metastatic breast carcinoma (MBC) (HER2CLIMB)

Hamilton E, Borges V, Murthy R, Anders C, Cameron D, Carey L, Müller V, Curigliano G, Gelmon K, Hortobagay G, Krop I, Loibl S, Pivrot X, Pegram M, Slamon D, Hurvitz S, Tsai M and Winer E. Sarah Cannon Research Institute, Nashville, TN; Tennessee Oncology, PLLC, Nashville, TN; University of Colorado Cancer Center, Aurora, CO; The University of Texas MD Anderson Cancer Center, Houston, TX; University of North Carolina at Chapel Hill, Chapel Hill, NC; Oncology, Edinburgh Cancer Centre Western General Hospital, Edinburgh, United Kingdom; University Medical Center Hamburg-Eppendorf, Hamburg, Germany; Drug Development, Istituto Europeo di Oncologia, Milano, Italy; British Columbia Cancer Agency, Vancouver Cancer Centre, Vancouver, BC, Canada; Dana-Farber Cancer Institute, Boston, MA; Medicine and Research, German Breast Group, Neu-Isenburg, Germany; Service Oncologie Medicale, CHU Besançon, Hôpital Jean Minjoz, Besançon, France; The University of California Los Angeles, Los Angeles, CA; U.C.L.A. School of Medicine, Los Angeles, CA; University of California, Los Angeles (UCLA), Los Angeles, CA and Virginia Piper Cancer Institute, Minneapolis, MN.

Body: Background: ONT-380 is a highly selective small molecule inhibitor of HER2 kinase with nanomolar potency. Unlike dual HER2/EGFR agents, it does not inhibit EGFR at clinically relevant concentrations, decreasing the potential for EGFR-related toxicities (severe skin rash and diarrhea). In preclinical studies, ONT-380 demonstrated synergistic activity with T, and was active in HER2+ models of brain metastases (mets). In a Phase 1b study, ONT-380 was combined with C and T in pts with HER2+ MBC previously treated with trastuzumab emtansine (T-DM1) and T. Objective responses were seen, including in pts with brain mets. The combination was well tolerated, with low rates of Gr 3 diarrhea at the recommended dose (300 mg PO BID, equivalent to the single agent MTD). Based on these data, ONT-380 is now being evaluated in a Phase 2 study in combination with C and T (HER2CLIMB).

Trial Design: The primary study objective is to assess the effect of ONT-380 vs. placebo given with C + T on progression-free survival (PFS) based on independent central review. Additional objectives include ORR, duration of response, clinical benefit rate, and safety. The study population includes adult pts with progressive HER2+ locally advanced or MBC who have had prior treatment with a taxane, T, pertuzumab and T-DM1 but not C or lapatinib. Pts with brain mets, including untreated or progressive mets, may be enrolled. 180 pts will be enrolled in North America and Europe. Pts are receiving C (1000 mg/mg² PO BID for 14 days of a 21-day cycle) and T (8 mg/kg IV loading dose; 6 mg/kg IV once every 21 days), and are being randomized in a 2:1 ratio to ONT-380 300 mg PO BID or placebo. Pts with isolated CNS progression may continue on study treatment after undergoing local CNS therapy. An independent Data Monitoring Committee is monitoring pt safety.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-10

Title: A phase 2 study of poziotinib in patients with HER2-positive metastatic breast cancer (MBC) who have received prior HER2 regimens for MBC

Lathrop K, Lucas J, Vacirca JL L, Bhat G, Choi MR and Naughton M. The University of Texas Health Science Center at San Antonio, San Antonio, TX; Marin Cancer Care, Greenbrae, CA; North Shore Hematology/Oncology, East Setauket, NY; Spectrum Pharmaceuticals, Irvine, CA and Washington University, St Louis, MO.

Body: Background: Poziotinib is a novel, oral, quinazoline-based pan-HER inhibitor that irreversibly blocks signaling through the epidermal growth factor receptor (EGFR) family of tyrosine-kinase receptors, including EGFR (HER1/ErbB1/EGFR), HER2 (ErbB2), and HER4 (ErbB4), as well as HER receptor mutations. This, in turn, leads to inhibition of the proliferation of tumor cells that overexpress these receptors. It is well established that breast cancers are associated with a mutation in, or overexpression of, members of the EGFR receptor family. The primary objective of this Phase 2 study is to evaluate the Objective Response Rate (ORR) of poziotinib in patients with human epidermal growth factor receptor 2 (HER2)-positive MBC. The secondary efficacy variables are Progression-Free Survival (PFS), Disease Control Rate (DCR), Overall Survival (OS), and Time to Progression (TTP).

Trial Design: This is a Phase 2, open-label, multicenter study to evaluate the efficacy, safety and tolerability of poziotinib in patients with HER2-positive MBC who have received at least 2 prior HER2- directed treatment regimens. Each treatment cycle is 21 days in duration. During each cycle, eligible patients receive 24 mg of poziotinib orally (as three 8-mg tablets) once daily for 14 days, followed by a 7 day treatment-free period.

Eligibility Criteria: Eligible patients are at least 18 years of age, have confirmed HER2 overexpression, adequate hematologic, renal and hepatic function, and have received at least 2 prior HER2-directed therapy regimens, including trastuzumab and trastuzumab emtansine (TDM-1). Patients are excluded if they have prior exposure to poziotinib, a history of congestive heart failure, left ventricular ejection fraction <50%, unable to take oral medications, or have conditions that cause malabsorption. A 30 day wash out period from previous chemotherapeutic or radiation therapies is required.

Statistical Methods: The purpose of this study is to evaluate the efficacy of poziotinib compared to the efficacy of other standard HER2-positive breast cancer treatments as reported in the literature. The ORR will be analyzed descriptively along with the 95% CI. The secondary efficacy variables will be analyzed descriptively.

Target Accrual: Approximately 70 patients. Enrollment began February 2016.

Contact Information: For more information or to refer a patient, email: spi-poz-201@sppirx.com or fax: 1-949-398-9711.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-03-01

Title: The UK LORIS trial: Randomizing patients with low or low intermediate grade ductal carcinoma in situ (DCIS) to surgery or active monitoring

Francis A, Bartlett J, Billingham L, Bowden S, Brookes C, Dodwell D, Evans A, Fairbrother P, Fallowfield L, Gaunt C, Hanby A, Jenkins V, Matthews L, Pinder S, Pirrie S, Rea D, Reed M, Roberts T, Thomas J, Wallis M, Wilcox M and Young J. University Hospital Birmingham, Birmingham, United Kingdom; Ontario Institute for Cancer Research, Toronto, Canada; Cancer Research UK Clinical Trials Unit (CRCTU), Birmingham, United Kingdom; University of Leicester, Leicester, United Kingdom; St James's Hospital, Leeds, United Kingdom; University of Dundee, Dundee, United Kingdom; Independent Cancer Patients' Voice, England, United Kingdom; SHORE-C, Brighton and Sussex Medical School, Brighton, United Kingdom; University of Leeds, Leeds, United Kingdom; King's College London, London, United Kingdom; Brighton and Sussex Medical School, Brighton, United Kingdom; Western General Hospital, Edinburgh, United Kingdom and Addenbrooke's Hospital, Cambridge, United Kingdom.

Body: Background: The independent review of the UK National Health Service Breast Screening Programme reported (The Lancet, Volume 380, Issue 9855, Page 1778, 17 Nov 2012) on the benefits and harms of breast screening. It concluded that breast screening saves lives and acknowledged the existence of overtreatment. It encouraged randomized trials to elucidate the appropriate treatment of screen-detected DCIS to gain a better understanding of its natural history. The LORIS trial addresses the possible overtreatment of low and low/intermediate grade screen-detected (low risk) DCIS by randomizing patients to standard surgical treatment or active monitoring, each with long term follow up.

Trial Design: LORIS is a phase III, multicentre, 2 arm study, with a built in 2 year Feasibility Phase, in patients confirmed to have low risk DCIS defined by strict criteria and determined by central pathology review. Patients will be randomized between standard surgery and active monitoring with annual mammography. Patients will be followed up for a minimum of 10 years.

Eligibility Criteria:

- 1) Female, age \geq 46 years
- 2) Screen-detected or incidental microcalcification (with no mass lesion clinically or on imaging)
- 3) Low risk DCIS on large volume vacuum-assisted biopsy, confirmed by central pathology review
- 4) Patient fit to undergo surgery
- 5) No previous breast cancer or ipsilateral DCIS diagnosis
- 6) Written informed consent

Specific Aims: The LORIS Trial aims to establish whether patients with newly diagnosed low risk DCIS can safely avoid surgery without detriment to their wellbeing (psychological and physical) and whether those patients that do require surgery can be identified by pathological and radiological means.

Primary endpoint: Ipsilateral invasive breast cancer free survival time

Secondary endpoints: Overall survival; mastectomy rate; time to mastectomy; time to surgery; patient reported outcomes; health resource utilisation and assessment of predictive biomarkers.

A digital image data repository and tissue bank will provide a prospective resource for both translational and imaging studies.

Statistical Methods: A total of 932 patients will be randomized to a non-inferiority design to test the null hypothesis that active monitoring of women diagnosed with low risk DCIS is not non-inferior in terms of ipsilateral invasive breast cancer free survival (iiBCFS) time compared to treatment with surgery. The iiBCFS time will be compared across the two arms on a per protocol and intent-to-treat basis, using a 1-sided ($\alpha=0.05$) log-rank test for non-inferiority. The iiBCFS rate is assumed to be 97.5% in the surgery arm at 5 years, utilizing 80% power to exclude a difference of more than 2.5% in the active monitoring arm.

Present Accrual and Target Accrual: 32 UK centres are open for the Feasibility Phase of the trial which is nearing completion. The web-based central pathology review process is functioning efficiently, with a one week maximum turn around. Registrations and sites randomizing patients are on or above target. Randomizations are currently approximately 70% of target. A total of 60 centres will open in the main trial.

Contact Information: For further information, please email the LORIS Trial Office LORIS@trials.bham.ac.uk.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-04-01

Title: Cambridge brain mets trial 1 (CamBMT1): A proof-of-principle phase 1b / randomised phase 2 study of afatinib penetration into brain metastases for patients undergoing neurosurgical resection, both with and without prior low-dose, targeted radiotherapy

Baird RD D, Ramenatte N, Watts C, Jonson A, Jones L, Biggs H, Harrison E, Oberg I, Bullen G, Williams M, Qian W, Gilbert F, Jodrell D, Caldas C, Karabatsou K, Dunn L, Jena R, Whitfield G, Chalmers A, Jefferies S and Price S. Cambridge Cancer Centre - Breast Cancer Research Unit, Cambridge, Cambridgeshire, United Kingdom; Cambridge Clinical Trials Unit – Cancer Theme, Cambridge, Cambridgeshire, United Kingdom; Cambridge Cancer Centre - Early Phase Clinical Trials Team, Cambridge, Cambridgeshire, United Kingdom; University of Cambridge, Cambridge, Cambridgeshire, United Kingdom; Neuro-Oncology, Addenbrooke's Hospital, Cambridge, Cambridgeshire, United Kingdom; Cancer Research UK Cambridge Institute - PK-PD Core Facility, Cambridge, Cambridgeshire, United Kingdom; University of Glasgow - Neuro-Oncology, Glasgow, United Kingdom; University of Manchester, Manchester Academic Health Science Centre, Christie NHS Foundation Trust, Manchester, United Kingdom and University of Cambridge - Cambridge Cancer Centre, United Kingdom.

Body: Background

Failure of drugs to cross the blood brain barrier (BBB) can be a major reason for treatment failure for patients with brain tumors. For most patients who don't respond to treatment, it is not known whether this is due to inadequate drug concentrations in the tumor, or due to drug resistance. Preliminary data suggest that low-dose radiotherapy may disrupt the BBB, and could facilitate increased drug delivery into brain tumors. Afatinib is a potent, irreversible inhibitor of EGFR / HER2 / HER4 and takes approximately 8 days to achieve steady-state concentrations in cancer patients.

Aims

CamBMT1 has been designed to investigate the delivery of afatinib into brain metastases and whether this might be enhanced by low dose-radiotherapy.

Patient Population

Key eligibility criteria

Patients with operable brain metastases from breast or lung primaries for whom neurosurgical resection would be standard of care, as determined by the local multi-disciplinary team. ECOG PS 0, 1 or 2.

Trial design

After a phase 1b safety run-in, the phase 2 part of the trial randomises patients (n=60) into 3 pre-operative arms:

Arm 1	afatinib alone for 11 days, then neurosurgery on day 12
Arm 2	afatinib for 11 days plus a single 2 Gy fraction on day 10, then neurosurgery on day 12
Arm 3	afatinib for 11 days plus a single 4 Gy fraction on day 10, then neurosurgery on day 12

Primary endpoint: to compare steady-state afatinib concentration in resected brain metastases, following afatinib administered alone, or in combination with radiotherapy (2 Gy or 4 Gy). Afatinib concentrations are measured in the resected brain metastases and in plasma.

Secondary endpoints: safety of afatinib administration in combination with radiotherapy; and multi-sequence MRI (optional) to detect changes in perfusion, vascular density, blood-brain-barrier permeability and interstitial pressure.

Exploratory endpoints: molecular profiling of resected brain metastases, for comparison with paired primary lung and breast cancers; the establishment and study of patient-derived xenografts.

Statistical methods

With 20 patients randomised in each of 3 arms in the phase 2 part of CamBMT1, the trial has a power of 84% at a significance level of 20% (one-sided) to detect an increase in afatinib concentrations with targeted radiotherapy, measured as a Cohen's D

(standardised mean difference) ≥ 0.5 .

Accrual

By the end of q2 2016, phase 1b had nearly completed enrolment. The randomised phase 2 part of CamBMT1 is due to open by q4 2016 at additional Experimental Cancer Medicine Centres.

Acknowledgments

CamBMT1 is funded by Cancer Research UK, the Brain Tumour Charity and Boehringer-Ingelheim.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-04-02

Title: NRG oncology/RTOG 1119: Phase II randomized study of whole brain radiotherapy with concurrent lapatinib in patients with brain met from HER2-positive breast cancer — A collaborative study of RTOG & KROG (NCT01622868)

White JR R, Moughan J, Kim IA, Peereboom DM M, De Los Santos JF F, Sperduto PW W and Mehta MP P. The Ohio State University, Columbus, OH; NRG Oncology Statistics and Data Management Center, Philadelphia, PA; Seoul National University Bundang Hospital, Seoul, Korea; Cleveland Clinic Foundation, Cleveland, OH; University of Alabama at Birmingham, Birmingham, AL; Metro-Minnesota CCOP, St. Louis Park, MN and University of Maryland, College Park, MD.

Body: Background: The addition of trastuzumab to cytotoxic chemotherapy has improved outcomes for patients (pts) with HER2+ breast cancer. Increased survival coupled with limited blood-brain barrier (BBB) penetration of trastuzumab may contribute to the increased incidence of brain metastases (mets) in these pts. Half of these pts die of intracranial disease progression rather than extracranial systemic disease. Therefore, strategies to improve survival must include increased CNS disease control. Lapatinib crosses the BBB & demonstrates modest activity against intracranial mets. Based upon preclinical data & phase I study results, it's hypothesized that lapatinib plus whole brain radiotherapy (WBRT) can improve the intracranial disease control compared to WBRT alone.

Trial design: A randomized phase II trial that will evaluate if there is a sufficient enough signal in improved 12-week complete response (CR) rate following WBRT with the addition of lapatinib vs. WBRT alone in pts with mets from HER2+ breast cancer to warrant a future phase III trial. An amendment to allow protocol RT to be delivered as WBRT or stereotactic radiosurgery (SRS) is in process.

Eligibility Criteria: Eligibility includes HER2+ breast cancer with at least one measurable, unirradiated parenchymal brain met (≥ 10 mm if solitary, & > 5 mm if multiple on enhanced MRI). The two populations targeted for accrual include pts with 1) newly diagnosed, multiple brain mets or 2) progressive brain mets after SRS or surgical resection of 1-3 mets. Pts are stratified by breast-specific graded prognostic assessment; use of non-CNS penetrating HER2 targeted therapy; & prior SRS or surgical resection. Non-CNS penetrating HER2 targeted therapy is permitted throughout the study, but pts not on trastuzumab, pertuzumab or any other breast cancer therapy at study entry are not permitted to begin this therapy while on protocol treatment, but may begin it 24 hours after its completion. Prior lapatinib is allowed, last dose > 21 days prior to study entry.

Specific aims: Primary objective is to determine if there is an increase in CR rate in the brain at 12 weeks post WBRT as determined by MRI scan of the brain, with the addition of lapatinib to WBRT vs. WBRT alone. Secondary objectives includes: CR rate at 4 weeks on MRI post WBRT, objective response rate on MRI at 4 & 12 weeks, evaluation of lesion specific MRI response rates; CNS progression-free survival rate, overall survival rate, & adverse event rates.

Statistical methods: The randomization of experimental & control arms is set as 1:1. With 114 eligible pts there will be 86% power to detect a 15% absolute increase in CR rate at a significance level of 0.10, using a 1-sided Z-test for 2 proportions. Targeted accrual is 143 accounting for up to a 5% ineligibility rate, 15% pts not evaluable for the primary endpoint due to death, pt withdrawal, or other reasons.

Activated: 7/26/2012. Present accrual (5-1-2016): 85. Targeted accrual: 143.

Contact Information:

Protocol: CTSU member web site <https://www.ctsu.org>. Enrollment: OPEN at <https://open.ctsu.org>. Supported by NCI U10 grants CA21661, CA180868, CA180822, CA37422 & UG1CA189867.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-04-03

Title: Randomized phase II study of induction bevacizumab, etoposide and cisplatin followed by whole brain radiotherapy (WBRT) versus WBRT alone in breast cancer with untreated brain metastases (A-PLUS)

Lu Y-S, Tseng L-M, Yu J-C, Rau K-M, Chao T-Y, Chen S-C, Chiu C-F, Chang Y-C, Chen TW-W, Lin C-H, Chang D-Y, Chao T-C, Huang S-M, Huang C-S and Cheng A-L. National Taiwan University Hospital, Taipei, Taiwan; Taipei Veterans General Hospital, Taipei, Taiwan; Tri-Service General Hospital, Taipei, Taiwan; Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan; Shuang-Ho Hospital, Taipei, Taiwan; Chang Gung Memorial Hospital, Taipei, Taiwan; China Medical University Hospital, Cancer Center, Taichung, Taiwan; Mackay Memorial Hospital, Taipei, Taiwan; Taipei Veterans General Hospital, Taipei, Taiwan and National Taiwan University Hospital, Taipei, Taiwan.

Body: Background For breast cancer (BC) patients with brain metastases (BM) who are not suitable for surgery/radiosurgery, whole brain radiotherapy (WBRT) remained the only standard treatment. Recently, we have demonstrated that bevacizumab preconditioning followed by etoposide and cisplatin (BEEP) is a highly effective treatment for BM of BC progressing from WBRT (Clin Cancer Res. 2015;21(8):1851). The CNS objective response rate is 77.1% according to volumetric criteria, and 60% according to RECIST 1.1. It has been demonstrated that enlarged brain tumor size is a predictor of WBRT failure. We hypothesized that, for BC with BM, induction BEEP treatment could decrease the size of brain metastases and thereby enhance effectiveness of WBRT. **Methods** This is a Phase II, randomized, open-labelled study (NCT02185352). Key inclusion criteria: BC with measurable brain metastatic tumor who had not received WBRT and not suitable for surgery or radiosurgery; KPS \geq 30%. Key exclusion criteria: patients who had leptomeningeal metastases; history of disease progression during prior cisplatin treatment. In the experimental arm, patients will be treated by induction BEEP for three cycles followed by WBRT. In the control arm, patients will receive upfront WBRT for brain metastases. The BEEP regimen consist of bevacizumab (15 mg/kg) on Day 1 and, with a 1 day window period, followed by etoposide (70 mg/m²/day, Day 2 to Day 4) and cisplatin (70 mg/m², Day 2), in a 21-day cycle. Stratification is based on the Graded Prognostic Assessment (GPA) score. Primary endpoint: brain-specific progression free survival (PFS) according to RECIST 1.1; key secondary endpoint: the 2-month brain-specific objective response rate (BS-ORR) of BEEP alone and WBRT alone. Other secondary endpoints include overall survival, extra-CNS tumor PFS, safety, time-to-improvement of neurological function, brain-specific PFS according to volumetric criteria, and BS-ORR. Approximately 126 patients will be 2:1 randomized. Multi-center recruitment is ongoing. To our knowledge this is the first randomized trial investigating a targeted therapy plus chemotherapy as induction regimen followed by WBRT as first line treatment for BC with BM.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-04-04

Title: A phase 2 randomized, double-blind, placebo-controlled trial of endocrine therapy ± radium-223 dichloride in HER2-negative, hormone receptor-positive breast cancer patients with bone metastases

Coleman RE E, Fried G, Petrenciuc O, Sawhney A, Li R and Rugo HS S. University of Sheffield, Weston Park Hospital, Sheffield, United Kingdom; Rambam Medical Center, Haifa, Israel; Bayer HealthCare Pharmaceuticals, Whippany, NJ and UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA.

Body: Background: Bone-metastatic breast cancer (MBC) treatment is limited. In a phase 2a study of bone-dominant MBC patients, radium-223, a first-in-class α emitter with targeted cytotoxic effect on bone metastases (mets), reduced bone biomarker levels with favorable safety (Coleman et al. *Breast Cancer Res Treat.* 2014).

Trial design: This study evaluates efficacy and safety of radium-223 versus placebo (pbo), each + endocrine treatment (ET), in patients with HER2⁻ estrogen receptor⁺ (ER⁺) bone-dominant MBC (NCT02258464). Patients receive (1:1) radium-223 50 kBq/kg IV or pbo q 4 wk (6 cycles) + ET + denosumab or bisphosphonates + best supportive care. Stratification is by geographic region (EU/N America vs Asia), number of prior ET lines (1 vs ≥ 2) for MBC, and number of prior skeletal-related events (SREs) (1 vs 2).

Eligibility criteria: Eligible patients are pre- or postmenopausal with HER2⁻ ER⁺ bone-dominant MBC and ≥ 2 bone mets or with soft tissue and/or visceral mets, and 1-2 prior SREs (external beam radiotherapy, pathologic bone fracture, spinal cord compression, orthopedic surgery); they have received ≥ 1 line of ET for MBC and are considered appropriate for further ET. Patients must have evaluable disease (RECIST 1.1), be taking bisphosphonates or denosumab for ≥ 1 month before study treatment, have an ECOG score 0-1, and have adequate hematologic, renal, and liver function. Patients must not have had visceral or brain mets or leptomeningeal disease, or need chemotherapy for MBC, and must not be suitable for everolimus for MBC. Patients are not eligible if they had prior radium-223 treatment or have untreated spinal cord compression.

Specific aims: The primary end point is SSE-free survival (SSE-FS). Secondary end points are radiologic progression-free survival; overall survival; times to opioid use, pain progression, and cytotoxic chemotherapy; pain improvement rate; and safety. Patients are assessed for efficacy and safety and are followed to SSE, radiologic progression, death, or withdrawal.

Statistical methods: Assuming 1-sided α 0.1, power 90%, ~ 119 SSE-FS events are needed for analysis. Time-to-event analysis will use a log-rank test, accounting for stratification. Kaplan-Meier estimates and survival curves will be given for each treatment group. Safety analyses will be descriptive.

Present and target accrual: Target accrual is ~ 227. Currently, 40 patients are randomized.

Contact Oana Petrenciuc, Bayer HealthCare Pharmaceuticals, oana.petrenciuc@bayer.com, for more information.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-04-05

Title: A phase 2 randomized, double-blind, placebo-controlled trial of radium-223 dichloride with exemestane and everolimus in patients with HER2-negative, hormone receptor–positive breast cancer and bone metastases

Rugo HS S, Drumea KC C, Campone M, Barnadas A, Petrenciuc O, Zhang A, Li R and Coleman RE E. UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; Rambam Medical Center, Haifa, Israel; Institut de Cancerologie de l'Ouest, Saint Herblain, France; Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; Bayer HealthCare Pharmaceuticals, Whippany, NJ and University of Sheffield, Weston Park Hospital, Sheffield, United Kingdom.

Body: Background: Treatment options for bone-dominant metastatic breast cancer (MBC) are limited. Radium-223, a first-in-class α emitter with a targeted antitumor effect on bone metastases (mets), was well tolerated and reduced bone biomarker levels in a phase 2 study in patients with bone-dominant MBC (Coleman et al. *Breast Cancer Res Treat.* 2014). In patients with HER2⁻ estrogen receptor⁺ (ER⁺) bone-dominant MBC, everolimus + exemestane (EVE+EXE) improved progression-free survival (PFS) versus EXE alone. Radium-223 combined with EVE+EXE may improve outcomes in patients with HER2⁻ ER⁺ bone-dominant MBC; this trial will evaluate efficacy and safety of radium-223 versus placebo in these patients (NCT02258451).

Trial design: Patients are randomized to receive (1:1) radium-223 (50 kBq/kg [55 kBq/kg after National Institute of Standards and Technology update] IV) or placebo \times 6 cycles q 4 wk + EXE (25 mg PO q d) + EVE (10 mg PO q d) plus best supportive care. EXE+EVE continues until disease progression or unacceptable toxicity. Stratification is by geographic region (EU/N America vs Asia), prior hormone therapy (1 vs \geq 2), and presence of visceral disease (yes vs no).

Eligibility criteria: Eligible patients are pre- or postmenopausal with HER2⁻ ER⁺ MBC and have \geq 2 bone mets or have soft tissue and/or visceral mets. Patients must have measurable disease per RECIST v1.1, \geq 1 prior line of hormone therapy for MBC, and 1-2 prior skeletal-related events; be on bisphosphonates or denosumab; and have an ECOG score of 0-1. Patients must have had no past or current need for chemotherapy for MBC, no unresolved spinal cord compression, and no prior EVE treatment.

Specific aims: The primary end point is symptomatic skeletal event–free survival (SSE-FS). Secondary end points are overall survival; times to opiate use, pain progression, and cytotoxic chemotherapy; radiologic PFS; and safety. Safety and efficacy are assessed every 4 weeks. Long-term safety is assessed until study termination.

Statistical methods: Assuming a 1-sided α of 0.1, 90% power, \sim 160 SSE-FS events will be required for the analysis. Efficacy will be analyzed by a stratified log-rank test. Safety analysis will be descriptive.

Present and target accrual: Estimated enrollment is \sim 311 patients. Currently, 74 patients are randomized.

Contact Oana Petrenciuc, Bayer HealthCare Pharmaceuticals, oana.petrenciuc@bayer.com, for more information.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-04-06

Title: NRG-BR002: A phase IIR/III trial of standard of care therapy with or without stereotactic body radiotherapy (SBRT) &/or surgical ablation for newly oligometastatic breast cancer

Chmura SJ J, Winter KA A, Salama JK K, Woodward WW W, Borges VF F, Al-Hallaq H, Martuszak M, Jaskowiak NT T, Milano MT T, Bandos H and White JR R. University of Chicago, Chicago, IL; NRG Oncology Statistics and Data Management Center, Philadelphia, PA; Duke University Medical Center, Durham, NC; MD Anderson, Houston, TX; University of Colorado, Denver, CO; University of Michigan, Ann Arbor, MI; Univeristy of Rochester, Rochester, NY and University of Ohio, Columbus, OH.

Body: Background:

The current standard of care for metastatic breast cancer patients (pts) is to deliver palliative chemotherapy, biologic &/or hormonal therapy when appropriate, with radiation &/or surgery reserved for the management of symptomatic or non-responsive (mets). For selected pts with limited metastatic (met) disease, mets-directed ablative therapy, with either surgical resection or high dose conformal radiotherapy (in addition to standard systemic therapies) to lung, liver, CNS adrenal, & multiple organs has been shown to result in long-term disease control numerically superior to systemic therapy alone. NRG-BR002 is a randomized Phase II trial to evaluate stereotactic body radiotherapy (SBRT) &/or surgical resection (SR) of all met sites in newly oligometastatic breast cancer in addition to standard systemic therapy.

Trial Design & Eligibility

For the Ph IIR, eligible breast cancer pts with biopsy confirmed metastatic disease to ≤ 2 sites will be randomized to receive either standard systemic therapy with mets directed therapy as needed (control arm) versus ablative therapy of all met sites with either body SBRT &/or surgical resection (SR) (per the treating physician discretion) to determine if there is an improvement median progression free survival (PFS). If this aim is met the trial continues as a Ph III to evaluate if SBRT/SR improves 5 year overall survival. Secondary aims include local control in the met site, new distant met rate, & technical quality. The primary translational endpoint tests whether < 5 CTCs (per 7.5ml of blood) is an independent prognostic (outcome) marker for improved PFS & OS in oligometastatic breast cancer.

Women are within 1 year of metastatic diagnosis, on first line systemic therapy without progression & the primary site disease is controlled. CNS mets are ineligible. ER/PR & HER-2 neu are required on either the primary or met site. Site radiation credentialing & a facility questionnaire are required. Randomization is to standard systemic therapy with local radiotherapy/ surgery for palliation when necessary vs ablative therapy of all mets with SBRT &/or SR.

Statistics:

For the Ph IIR portion to detect a signal for improved median PFS from 10.5 months to 19 months with 95% power & accounting for ineligible/lost pts, 146 pts will be required. For the Ph III, an additional 246, for a total of 402 pts will be required to definitively determine if ablative therapy improves 5-year overall survival from 28% to 42.5% (HR=0.67), with 85% power & a 1-sided type I error of 0.025. For the translational research, the number of pts accrued in the Ph IIR & Ph III portions will provide sufficient power $\geq 91\%$ to detect whether < 5 CTC's is a prognostic marker for improved PFS & OS.

Present Accrual & Target Accrual

NRG BR002 activated 12/24/2014 with a target accrual of 146 pts for the Ph II component & 256 additional for the Ph III.

Contact Information:

Protocol: CTSU member web site <https://www.ctsuo.org>. Enrollment: OPEN at <https://open.ctsuo.org>.

Support:

Supported by NRG Oncology grants U10CA180868 & U10CA180822 from the National Cancer Institute (NCI).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-04-07

Title: VICTORIANE: A randomized phase 3 study assessing the addition of oral vinorelbine to aromatase inhibitors for the treatment of patients with metastatic breast cancer

Villanueva CB B, Barthelemy P, Ferrero JM, Jacquin JP, Bonnetain F, Mansi L and Pivot X. University Hospital of Besançon, Besançon, France; University Hospital of Strasbourg, Strasbourg, France; Centre Lacassagne de Nice, Nice, France; Institut de Cancérologie Lucien Neuwich, Saint Priest en Jarez, France and Université de Franche Comté, Besançon, France.

Body: Background : The addition of anti-proliferative agents such new inhibitors of CDK4/6 to hormonotherapy have significantly improved the efficacy in patients with metastatic breast cancer (MBC). Oral vinorelbine provided the opportunity to give new schedules of chemotherapy with favourable tolerability and prolonged exposure. The present trial was designed to evaluate the clinical effects of the addition of oral vinorelbine to aromatase inhibitors in first line treatment for MBC.

Methods : In this phase III, randomized, prospective, open trial named VICTORIANE (NCT02730091), postmenopausal women with HR+/HER2- with MBC are randomized (1:1) between letrozole or anastrozole once a day (Arm A) versus oral vinorelbine 50 mg three times a every week with letrozole or anastrozole (Arm B). Continuous daily schedule (days 1-28 of each 28 days cycle) is planned until disease progression or discontinuation for other reasons. A minimisation algorithm was used stratifying treatment allocation according to the existence of visceral metastases, prior adjuvant hormonal treatment and centers. The study is conducted in compliance with the principles of good clinical practice and the declaration of Helsinki.

Key inclusion criteria include, histologically proven HER2-negative and Estrogen Receptor-positive breast cancer, metastatic setting, no prior systemic anti-cancer therapy for MBC, recurrence after prior hormonal therapy in the adjuvant setting is allowed if disease free interval is greater than 24 months from the completion of treatment, normal liver, bone marrow and renal functions, *Performans status* greater than 2. Non inclusion criteria include symptomatic visceral disease, or disease burden precluding endocrine therapy, and prior therapy with vinorelbine.

The primary endpoints is progression-free survival (PFS; local assessment, RECIST v1.1) and secondaries endpoints are overall survival, health-related quality of life, overall response rate and safety. Analysis of the primary endpoint will be performed with a stratified log-rank test (95% confidence interval). A 30 percent reduction in the risk of events (Hazard Ratio (HR) = 0.70) was assumed under H1 in the arm B. This reduction was estimated based on an absolute gain of 3.857 months in terms of median PFS, from 9 months (arm A) to 12.857 months (arm B). Global recruitment of the planned 340 pts is ongoing and should be completed in march 2018. Final results will be expected in 2019.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT1-04-08

Title: Phase 3 study of etirinotecan pegol versus treatment of physician's choice in patients with metastatic breast cancer who have stable brain metastases previously treated with an anthracycline, a taxane, and capecitabine

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Body: Background: Etirinotecan pegol (EP) is a next-generation topoisomerase I inhibitor-polymer conjugate that provides continuous exposure to SN-38. A mouse model of brain metastases demonstrated high penetration and retention of SN-38 in CNS lesions, resulting in decreased size of CNS lesions and improved survival at concentrations clinically achieved at the recommended dose of EP in patients (pts) (Adkins et al, BMC Cancer 2015). A previous phase 3 trial comparing single-agent EP to treatment of physician's choice (TPC) was conducted in 852 pts with advanced breast cancer (BC) (Perez et al, Lancet Oncol 2015). Although the primary efficacy endpoint of improved survival was not met (HR 0.087; p value = 0.08), a subset of 67 pts who entered the study with stable brain metastases demonstrated improved overall survival (HR 0.51 [95% CI 0.304 – 0.858]; p < 0.01). A phase 3 trial has therefore been designed for this population of high unmet medical need. **Trial Design:** Pts with MBC with stable brain metastases will be randomized 1:1 to receive either single-agent EP or TPC in an open-label, randomized, multicenter Phase 3 study. **Key Entry Criteria:** Adults, with ECOG PS 0 or 1 with adequate liver, kidney and marrow function. All pts must have received prior therapy with an anthracycline, a taxane, and capecitabine (ATC) (these drugs may have been administered in the neo/adjuvant or locally advanced/metastatic setting); pts must have had 1 prior cytotoxic regimen for MBC (triple negative BC); 2 prior cytotoxic regimens and 1 prior hormone therapy (hormone-receptor+ BC); or 2 prior cytotoxic regimens and 1 prior HER2-targeted therapy (HER2+ BC). Pts are required to have undergone definitive local therapy of brain metastases (either whole brain radiation; stereotactic radiation or surgical resection); signs and symptoms of brain metastases must be stable with steroids either unchanged or decreasing for ≥ 7 days prior to randomization. Prior toxicities must have resolved to \leq Grade 1 (except sensory neuropathy \leq Grade 2 and complete resolution of prior diarrhea). **Methods:** Primary efficacy endpoint is OS. Key secondary endpoints: ORR by RECIST v1.1, clinical benefit rate (ORR+SD ≥ 6 months), PFS by RANO-BM and QoL. EP is given IV at 145 mg/m² over 90-min every 21 days without premedications. Pts randomized to TPC must receive 1 of the following: eribulin, ixabepilone, vinorelbine, gemcitabine, paclitaxel, docetaxel or nab-paclitaxel (the agent must be available at the treating institution). Pts are stratified by region, ECOG PS and receptor status (TNBC, HER2+ or HR+/HER2-). **Target Accrual:** ~350 pts will be randomized to obtain the number of deaths required at 90% power to detect a statistically significant improvement in OS; 1 interim analysis will occur when 50% of the deaths are reported (130 events). PK sampling is performed in a subset of pts; UGT1A1 testing will occur in pts randomized to EP. Plasma ctDNA will be assessed at baseline for potential predictive markers of efficacy. Enrollment is expected to open in 2016.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-01

Title: Circulating tumor cell number and CTC-endocrine therapy index predict clinical outcomes in ER positive metastatic breast cancer patients: Results of the COMETI Phase 2 trial

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Body: Introduction: Only half of hormone receptor positive (HR+) metastatic breast cancer (MBC) patients (pts) benefit from endocrine therapy (ET). Circulating tumor cells (CTC) are prognostic in pts with MBC using CellSearch® technology. The CTC-endocrine therapy index (CTC-ETI) provides semi-quantitative analyses of CTC-ER (estrogen receptor), BCL2, HER2, and Ki67 expression. We hypothesized that CTC-ETI high (elevated CTC number and/or low expression of ER and BCL2, and high expression of HER2 and Ki-67) might predict resistance to ET in a prospective, multi-institutional clinical trial: COMETI-P2-2012.0 (NCT01701050).

Methods: 121 pts with ER+, HER2 negative (-), and progressive MBC after one or more lines of ET or within 12 months (mos) of completing adjuvant ET, who were initiating a new ET, were enrolled after informed consent. CTC and CTC-ETI were determined as previously reported (Paoletti C *et al*, CCR 2015) at baseline (BL), 1, 2, 3, and 12 mos, and/or at the time of progression. Imaging was performed every 3 mos. Association of CTC levels and CTC-ETI with patient outcomes (progression free survival (PFS); rapid progression (RP) defined as progression within 3 mos) was assessed using logrank and Fisher's exact tests. Trial design estimated 85 PFS and 51 RP events, providing >90% power (2-sided $\alpha=0.05$); pts with unsuccessful BL CTC-ETI or ineligible were unevaluable. Only baseline (BL) data are reported in this abstract.

Results: 32% of enrolled pts had progression within 12 mos of completing adjuvant ET, whereas 40%, 20%, and 8% had 1, 2, ≥ 3 lines of ET for MBC. CTC-ETI was successfully determined in 93% of pts (90% CI, 88% to 97%). CTC were ≥ 5 CTC/7.5 ml whole blood in 37/108 (34%) pts evaluable for clinical validity. Elevated CTC was associated with worse PFS (median (m) PFS: 3.3 vs. 5.9 mos; $P<0.01$). Low, intermediate, and high CTC-ETI were observed in 75 (69%), 6 (6%), and 27 (25%) pts, respectively. CTC-ETI was associated with PFS (logrank $P<0.01$): pts with low, intermediate, and high CTC-ETI had mPFS of 5.7, 8.5, and 2.8 mos, respectively. In the 96 pts eligible for determination, elevated CTC was associated with RP, (65.6% vs. 42.2%; $P=0.05$) as was CTC-ETI ($P=0.003$): 79.2% (95% CI, 57.8% to 92.9%) of pts with high CTC-ETI had RP versus 41.2% (95% CI, 29.4% to 53.8%) with low CTC-ETI; in the small group with intermediate CTC-ETI 1 of 4 pts (25%) had RP.

Conclusions: In this multi-institutional, prospective study, CTC-ETI was accurately determined, confirming the previously established analytical validity of the assay, meeting the primary objective of the trial. Elevated CTC and CTC-ETI high compared to low were associated with poor outcomes to ET. CTC-ETI distribution resulted in a small number of patients assigned to the intermediate group, restricting our ability to associate this group with outcomes. These results suggest that CTC-biomarker phenotype and enumeration have clinical validity. CTC-ETI may identify ER+ HER2- MBC pts who are unlikely to benefit from ET and might be better treated with ET in combination with other therapies or proceed to chemotherapy. Further analyses including CTC-ETI at serial time points during ET are planned.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-02

Title: Circulating tumor cells (CTC) and endothelial cells (CEC) prognostic value in HER2 negative metastatic breast cancer patients treated with first line weekly paclitaxel and bevacizumab: First results of a prospective cohort from the French Breast Cancer InterGroup Unicancer (UCBG): COMET study

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Body: Background: increased levels of circulating tumor cells (CTC) are associated with worse progression-free survival (PFS) and overall survival (OS) in patients (pts) with metastatic breast cancer (MBC). It has been hypothesized that bevacizumab could modify CTC prognostic value. CEC variations to predict benefit of anti-angiogenic treatment is still controversial. Predictive markers for response to bevacizumab combined to chemotherapy in MBC remains a clinical unmet need.

Patients & methods: The French cohort COMET is a prospective study including first line HER2 negative patients (pts) receiving weekly paclitaxel and bevacizumab according to EMA approved combination. The aim of this cohort is to evaluate clinical, biological and radiological parameters associated with pts outcome (CTC, CEC, serum markers, ctDNA, pharmacogenomic polymorphisms, metabolomic parameters, visceral fat assessed by initial CTscan, serum estradiol level, and quality of life). We present here the first planned analysis on 203 pts evaluated for CTC and CEC using the FDA cleared CellSearch method.

Results: For CTC substudy, 211 patients were included from 09/2012 to 5/2014. Median follow-up is 24 months. Median PFS was 10 months (CI95 9-12) and response rate was 57%. Median OS was not reached. 203 patients were evaluable for both CTC and CEC at baseline and first day of second cycle of CT (D1C2). At baseline, 97/203 (48%) pts had ≥ 5 CTC (median 4 (range 0-30,000)). Median number CEC was 21 (0-2231) at baseline and 22 (1-881) at D1C2. LDH, CEA, CA15.3 and CYFRA 21 were above normal at baseline in 44%, 46%, 73% and 71% of the cases respectively. CTC level was not correlated with any patients' characteristics except a number of metastatic site >3 . After one cycle of chemotherapy (D1C2) 37 pts (22%) had still ≥ 5 CTC: 36 pts with initial high level and only one patient with low CTC at baseline had increased CTC above 5. Prognostic factors for PFS at univariate analysis were visceral disease, number of metastatic sites (> 3), triple negative status, LDH, CTC level at baseline and CTC level after one cycle of chemotherapy (D1C2). None of serum marker nor CEC level at baseline or any variations had prognostic value. In multivariate analysis for PFS, CTC level after one cycle of chemotherapy predicts poor outcome

Table 1 Multivariate analysis for PFS

Pts' characteristics	n	RR	CI 95%	p-value
Number of metastatic sites				
<3 sites	97	1		
≥ 3 sites	100	1.65	[1.13 ; 2.41]	0.010
CTC D1C2				
< 5	132	1		
≥ 5	37	2.17	[1.43 ; 3.29]	<0.001
Hormonal status				
Luminal (HR+)	153	1		
Triple negative	37	2.86	[1.85 ; 4.54]	<0.001

Conclusion: We confirm in a large prospective series the lack of clinical validity of CEC to predict response to an antiangiogenic based treatment in MBC. A persistent elevated level of CTC after just one cycle of chemotherapy is a very strong and independent marker of poor outcome in a homogeneously bevacizumab-treated cohort of MBC patients. This marker could be used to stopped earlier an inefficient and costly treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-03

Title: Dynamics of circulating tumor cells during the course of chemotherapy and prognostic relevance across molecular subtypes in high-risk early breast cancer patients – Results from the adjuvant SUCCESS A trial

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Body: Background: The presence of circulating tumor cells (CTCs) before chemotherapy is known to be associated with reduced disease free survival (DFS) and overall survival (OS) in early breast cancer (EBC). In addition, recent findings suggest that CTCs persisting after adjuvant chemotherapy indicate poor prognosis. In an explorative analysis of the SUCCESS A trial, we evaluated the prognostic relevance of changes in CTC counts during the course of adjuvant chemotherapy across molecular subtypes to assess whether the prognostic role of persisting CTCs varies according to tumor biology.

Methods: The SUCCESS A trial is a phase III study, where patients with high-risk EBC (stage pN1-3 or pT2-4 or grade 3 or age ≤ 35 or hormone-receptor negative) were randomized to adjuvant chemotherapy with 3 cycles of epirubicin-fluorouracil-cyclophosphamide followed by either 3 cycles of docetaxel or 3 cycles of gemcitabine-docetaxel. CTC enumeration was performed before and after chemotherapy using the FDA-approved CellSearch® System (Janssen Diagnostics, LLC), and CTC positivity was defined as ≥ 1 CTC in 23 ml blood. Molecular subtypes were defined as luminal A like (hormone-receptor positive, grading 1 or 2), luminal B like (hormone-receptor positive, grading 3), triple-negative or HER2-positive. Patient outcome in terms of DFS and OS was analyzed using univariate log-rank tests and Cox regression models (median follow-up time 65.2 months).

Results: Data on both molecular subtypes and CTC status before and after chemotherapy were available for 1485 (39.6%) of 3754 patients randomized. This cohort contained 577 (38.9%) luminal A like, 236 (15.9%) luminal B like, 379 (25.5%) HER2-positive and 293 (19.7%) triple negative tumors. Overall, 917 (61.8%) patients were CTC negative before and after chemotherapy (neg/neg), 260 (17.5%) patients had a negative CTC status before and a positive CTC status after chemotherapy (neg/pos), 229 (15.4%) patients converted from positive to negative CTC status (pos/neg), and 79 (5.3%) patients were positive for CTCs at both time points (pos/pos). There were significant differences in DFS and OS among these four groups in patients with luminal A like tumors (log rank test, both $p < 0.003$) and patients with luminal B like tumors (log rank test, both $p < 0.001$). In both patients with luminal A like or luminal B like tumors, persistently CTC positive patients had the worst outcome (relative to persistently CTC-negative patients) in terms of DFS and OS. In contrast to luminal-like tumors, no significant differences with regard to DFS or OS were found among the four groups (neg/neg, neg/pos, pos/neg, pos/pos) in patients with HER2-positive or triple-negative tumors (log rank test, all $p > 0.13$).

Conclusion: The presence of CTCs both before and after adjuvant chemotherapy was associated with poor survival in luminal A like and luminal B like tumors, but not in HER2-positive or triple-negative tumors. Further research is needed to evaluate the effect of chemotherapy on CTC prevalence in different molecular subtypes of EBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-04

Title: Comprehensive genomic characterization of circulating tumor cells (CTCs) in metastatic breast cancer (MBC) sheds light on the biology of blood-borne metastasis

Magbanua MJ, Hauranieh L, Roy R, Wolf D, Benz S, Vaske C, Pendyala P, Sosa E, Scott J, Lee JS, Ordonez A, Ho B, Solanki T, Vantveer L, Rugo H and Park J. University of California San Francisco and NantOmics.

Body: Background: CTCs offer a relatively non-invasive source of metastatic tissue for molecular analysis. To elucidate the underlying biology of blood-borne metastasis, we profiled CTCs from MBC patients (pts).

Methods: CTCs were isolated by IE/FACS (immunomagnetic enrichment/fluorescence-activated cell sorting). Expression of 64 cancer-related genes in CTCs was analyzed via microfluidic-based multiplex QPCR. Genome-wide copy number (CN) analysis by array comparative genomic hybridization (ACGH) was performed on CTCs isolated from the same tumor-enriched blood samples. The Illumina platform was utilized for next generation sequencing and data was analyzed using NantOmics analysis pipeline and Nexus 8.0 software. Mutations were confirmed by Sanger sequencing or by digital droplet PCR.

Results: Expression profiles of CTCs from 105 MBC pts clustered away from those of blood, indicating high-purity isolation of CTCs by IE/FACS. In addition to *EPCAM*, tumor-related genes, e.g., *CCND1*, *MUC1*, and *TTF3* were upregulated in CTCs. Approximately 70% of the CTC samples were considered ER-positive, of which 47% were ER+HER2+, and 22% ER+HER2-. Among the ER+HER2- samples, about two-thirds (68%) had low proliferative (*MKI67*) status. HER2-positive and triple-negative CTCs accounted for 27% and 30% of the samples, respectively. Furthermore, 30% of the samples were assigned to luminal A, 6% to luminal B, 13% to Her2-enriched, 33% to basal-like, and 12% to normal-like subtypes. Expression profiling of CTCs in 74 serial blood samples from 28 pts showed fluctuations in expression at the gene-level, while subtype calls were mostly consistent across time points.

CTCs from 49 of the 105 pts analyzed by ACGH revealed numerous genomic aberrations such as 1q/8q gains and 8p/16q losses, consistent with breast cancer origin. CN profiles grouped into three major clusters: CTCs exhibiting low genomic instability, 8q gain, and 1q gain/11q loss. *ERBB2* and *CCND1* were upregulated in CTCs showing increased genomic alterations.

Changes in ER (n=102) and HER2 (n=130) status between CTCs and matched primary tumors (PT) were observed in 27% and 23% of the pts, respectively, indicating that biomarker status may change during disease progression.

Comparative analysis of CN data from low-pass whole genome sequencing (WGS) of CTCs vs. matched PTs (n=7 pairs) demonstrated clonal-relatedness as well as some genetic divergence. WGS (38x) and whole exome sequencing (140x) analysis of CTCs from an index pt diagnosed with invasive lobular carcinoma detected numerous genomic aberrations, including a copy loss and a frameshift mutation in E-cadherin (*CDH1*). Interestingly, analysis of CN and mutation data revealed that CTCs were more closely related to the lymph node metastases than to the PT. Single-cell sequencing of CTCs revealed uniformity in genome-wide CN alterations, while cell-to-cell heterogeneity was observed only when single-cell expression profiles were analyzed.

Conclusions: Comprehensive molecular characterization provided novel insights into the biology of breast CTCs. Further CTC profiling may open avenues for discovery and development of novel biomarkers for personalized medicine and strategies to prevent metastasis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-05

Title: Prognostic values of circulating tumor cells (CTC) and cancer associated macrophage-like cells (CAML) enumerations in metastatic breast cancer: The role for innate immunity in the metastatic process

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Body: Background: The enumeration of circulating tumor cells (CTCs) using the CellSearch assay is a well-established prognostic and predictive marker for metastatic breast cancer (MBC). However, additional prognostic markers are lacking in patients with ≥ 5 CTCs in 7.5 ml of blood. Tumor-associated macrophages (TAMs) are derived from circulating monocytes or tissue-resident macrophages. TAMs have a controversial role in metastasis and anti-tumor processes. Recent studies showed that circulating cancer associated macrophage-like cells (CAMLs) are specialized phagocytic myeloid cells and found in the peripheral blood of patients with solid tumors including breast cancer, but not in healthy individuals. The presence of CAMLs may indicate the activation of innate immunity in cancer patients. The function and prognostic value of CAMLs in MBC is unknown. In the current study, we measured CTCs and CAMLs on the CellSearch™ platform and investigated their prognostic values in MBC.

Methods: Peripheral blood samples from 127 stages IV breast cancer patients were collected at baseline before starting first-line therapy. The detection and enumeration of CTCs and CAMLs in 7.5 ml blood sample were performed on the CellSearch™ system. CTCs were identified by cytokeratins (CK-8, 18, and 19) positive and CD45 negative staining. CAMLs were defined by positive staining for cytokeratins and CD45 (*Adams et al, PNAS, 111(9):3514-9, 2014*). CTCs and CAMLs enumeration in associations with the progression-free survival (PFS) and overall survival (OS) of patients were evaluated using Kaplan Meier curves and Cox proportional hazards modeling.

Results: The image review of CAMLs by using CellSearch analysis showed heterogeneous morphological phenotypes. CAMLs are large cells presenting enlarged nuclei or multiple individual nuclei, and both cytokeratin and CD45 positive with diffused cytoplasmic staining. Among the 127 MBC patients, 38 (29.9%) had elevated CTCs (≥ 5 CTCs), and 21 (16.5%) had at least one CAML detected. Patients with CAMLs had a significantly increased PFS ($p=0.0374$) and OS ($p=0.0042$), compared to patients without CAMLs at baseline. Patients with elevated baseline CTCs and CAMLs had worse PFS with a hazard ratio (HR) of 4.04 (95% CI 2.16 -7.56, $P<0.0001$), compared to patients with < 5 CTCs and without CAMLs. The combined analysis of baseline CTCs enumeration and CAMLs showed similar effect on patient OS. Compared to patients with < 5 CTCs and without CAMLs, patients with < 5 CTCs and with CAMLs, patients with ≥ 5 CTCs and without CAMLs, and patients with ≥ 5 CTCs and with CAMLs, had an increasing trend of death risk, with an HR of 2.66 (95% CI 0.53-13.21), 6.14 (2.10-17.92), and 9.13 (3.05-27.37), respectively (p for trend < 0.0001).

Conclusion: Baseline enumerations of both individual CTCs and CAMLs are feasible and increase our ability to accurately predict outcome in MBC patients. Evaluation of CAMLs in peripheral blood may be a marker of innate immunity and provide additional prognostic values for MBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-06

Title: Ki67-positive CTCs are associated with early disease relapse in patients with early breast cancer undergoing adjuvant chemotherapy

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Body: Background: The determination of Ki67 in the primary tumor has prognostic value in early breast cancer (BC). We evaluated Ki67 expression in circulating tumor cells (CTCs) from patients with early BC undergoing adjuvant chemotherapy and correlated Ki67 positivity with patient outcome. **Methods:** Ki67 expression in CTCs was evaluated by immunofluorescent analysis in paired blood samples of patients with early BC (n=166) obtained before and after adjuvant chemotherapy. Ki67 expression was also evaluated in CTC-positive patients at 6 - 24 months after the end of chemotherapy (n=31). Cytospins of peripheral blood mononuclear cells were double stained with A45-B/B3 cytokeratin and Ki67 antibodies. The proliferation index (PI) of CTCs was defined as the ratio of Ki67-positive CTCs/total CTCs. **Results:** CTCs were detected in 53 (32%) patients before and/or after chemotherapy. Ki67-positive [Ki67(+)] CTCs were identified in 79% of CTC-positive patients, 25% presenting exclusively Ki67(+) CTCs and 21%, exclusively Ki67(-) CTCs. The mean value of Ki67(+) CTCs/patient remained unchanged pre- and post-chemotherapy [(mean±SE): pre- vs post-chemotherapy 2.5±0.7 vs 4.2±2, respectively; p= 0.900]. Similarly, the PI among the total CTCs detected pre- and post-chemotherapy was 59% and 60%, respectively. Ten (19%) of 53 CTC-positive and 9 (8%) of 113 CTC-negative patients relapsed (p = 0.039). In addition, all CTC-positive patients who relapsed harbored Ki67(+) CTCs before and/or after chemotherapy. Interestingly, 70% of them experienced early disease recurrence, ranging from 6-29 months after the initiation of adjuvant chemotherapy. Furthermore, 38.5% of patients with exclusively Ki67(+) CTCs relapsed compared to none among patients with exclusively Ki67(-) CTCs (p = 0.041). Of the 31 CTC-positive patients evaluated during follow-up, 39% remained CTC-positive. However, only 33.3% of them harbored Ki67(+) CTCs, 8.3% had exclusively Ki67(+) CTCs and 66.7% exclusively Ki67(-) CTCs. The mean value of Ki67(+) CTCs/patient was significantly reduced on the follow-up samples [(mean±SE): follow-up vs pre-chemotherapy, 1.35±1.3 vs 2.5±0.7, respectively; p=0.014 and follow-up vs post-chemotherapy, 1.35±1.3 vs 4.2±2, respectively; p= 0.026]. **Conclusions:** Ki67 expression on CTCs is predictive of early relapse in patients with early BC. Ki67 expression is not decreased by adjuvant chemotherapy, whereas it is reduced early during follow-up, possibly due to adjuvant hormone therapy and/or anti-HER2 therapy. The above results suggest that additional therapy is needed for patients with early BC and Ki67(+) CTCs to prevent early disease recurrence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-07

Title: Maintenance of genomic integrity in dormant circulating tumor cells

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Body: More than 67% of deaths in breast cancer patients occur after the initial 5-year survival period while residual disease can be dormant for periods longer than 20 years. Patients are asymptomatic because circulating tumor cells (CTCs) remain dormant and are undetectable by current clinical tools. Dormant CTCs may retain their long-term tumor-initiating (LTI) potential by adhering to their original genome, unlike rapidly cycling cancer cells that are known to have increased genomic instability. We hypothesized that hyperactive mechanisms of DNA repair preserve the genomic make-up of dormant CTCs allowing them to retain their LTI potential, ultimately causing disease relapse.

We isolated and characterized breast cancer CTCs by multiparametric flow cytometry and DEPArray™. Individually isolated breast cancer CTCs had a large proportion (>40%) of dormant (Ki67-/PCNA-) cells. Dormant CTCs had a lower incidence of double-strand DNA breaks (DSB) than proliferating cells as assessed by the phosphorylation status of Serine139 on gamma H2AX. This observation was further validated in a panel of eight genetically distinct breast cancer cell lines. Second, to understand whether dormant cells are inherently more resistant to DSB, we induced DSB in breast cancer cells by UV radiation and bleomycin treatment, and measured residual DSB at regular intervals. Results showed that besides being more resistant to DSB *de novo*, dormant breast cancer cells were also more efficient repairing their DNA. There are two distinct phases of DSB repair - early [within 2 hours of DSB using Non-Homologous End Joining (NHEJ) methods] and late [evident after 24 hours using Homologous Recombination (HR)]. Unlike proliferating (S-G₂M) cells, dormant (G₀) cells lack the sister chromatid and repair their DNA exclusively by NHEJ methods. Therefore, and third, we investigated key players of the NHEJ pathway and examined their roles in maintaining genomic integrity. We found that the human telomere-associated protein RIF1, a mediator of alternative NHEJ, was significantly up-regulated in a dormant CTC subset. Dormant sub-populations of breast cancer cells confirmed RIF1 foci formation in areas of DNA damage. Fourth, mis-sense mutation of RIF1 in CAMA-1 cells (Δ RIF1 E1598K) as well as shRNA mediated RIF1 knockdown in HCC1954 and ZR-75-1 cell lines attenuated resistance of the dormant subset to UV and bleomycin treatment. Finally, RIF1 knockdown activated both p38 and pERK pathways albeit to varying degrees in multiple cell lines resulting in metastatic inefficiency in xenograft and syngeneic mouse models.

Collectively, these findings suggest that RIF1 may play functional roles in maintaining the genomic integrity of dormant CTCs and be a potential biomarker of breast cancer CTC survival while in circulation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-08

Title: The evolution of breast cancer circulating tumor cells mediating brain metastasis

Boral D, Vishnoi M, Liu HN N, Yin W, Hong DS S, Scamardo AT T and Marchetti D. Houston Methodist Research Institute, Houston, TX and University of Texas M D Anderson Cancer Center, Houston, TX.

Body: Multiple studies concur that CTCs - the “seeds” of fatal metastasis - intravasate into the bloodstream throughout the early stages of cancer promoting generation of micro-metastatic reservoirs, some of which ultimately evolve to metastatic tumors. However, while it may take decades for CTCs to progress from cells in circulation to clinically detectable metastases, once detected, the tumor usually grows at an exponential rate. This difference in growth dynamics at two ends of the metastatic spectrum should also be reflected in the behavior of CTCs shed from these organ sites. Having a focus towards breast cancer brain-colonizing CTCs and their properties, we hypothesized that a gradual evolution of dormant CTCs into mitotically active CTCs with brain-metastatic potency is central to the progression of primary breast cancer to brain metastasis.

We isolated CD44⁺/CD24⁻ (stem-like) and Pan-Cytokeratin⁺ (epithelial) breast cancer CTCs from peripheral blood of two groups of breast cancer patients – five with brain metastases (BCBM) (MRI-detectable) and five without brain metastases (No BCBM). CTCs were isolated by multi-parametric flow cytometry and the expression of CTC-specific antigen was confirmed by immuno-cytochemistry and DEPArray™ platform. Intra-cellular flow cytometry showed that No BCBM patients had 3-fold higher mitotically dormant (Ki67⁻) CTCs compared to BCBM patients. Whole genome expression arrays demonstrated low generalized transcriptional activity in CTCs suggestive of mitotic/metabolic dormancy. Comparison of CTC transcriptomes with ER⁺/PR⁺, HER2⁺ and triple negative breast cancers identified a unique CTC gene signature valid not only across the three molecular subtypes but also independent of the molecular subtype patients were initially diagnosed as. Pathway analyses predicted increased activation of pluripotency-related pathways along with decreased protein translational machinery and proliferative pathways in CTCs. Further, to dissect mechanisms of metastatic reactivation in the brain microenvironment, we compared the CTC transcriptome in BCBM vs No BCBM patients and identified a 126 gene signature potentiated in BCBM CTCs. Subsequent pathway analyses revealed upregulation of known CTC pathways like Notch along with novel hematopoietic and immune evasion networks. Cellular and functional annotations of cell migration and chemotaxis were significantly activated in BCBM along with pro-inflammatory (TNF, IL1 β , NF- κ B), immunomodulatory chemokines (CXCL8, CXCR4, CD86) and mitogenic growth factors (PDGF-BB). Lastly, lack of cell surface expression of Urokinase Plasminogen Activator Receptor (uPAR) and integrin beta-1 (int- β 1) were used to identify CTCs in a state of metastatic dormancy. Whole genome sequencing showed significantly higher incidence of genomic mutations in proliferating CTCs compared to their dormant counterparts.

We provide first time evidence of signaling pathways keenly implicated in breast cancer CTC biology based on comprehensive analyses of CTC transcriptomes. We discovered the existence of CTCs in a state of metabolic/mitotic dormancy and identified genomic instability as a switch for CTCs to revert to their proliferative phenotype.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-09

Title: Comparison of HER2, estrogen and progesterone receptor expression profiles of primary tumor, synchronous axillary lymph node metastases and circulating tumor cells in early breast cancer patients

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Body: Background: Targeted systemic therapy in early breast cancer with synchronous lymph node metastasis is currently based on the expression of the hormone receptors (ER/PR $\geq 1\%$) and overexpression of HER2 in the primary tumor. However, the expression of these predictive markers on lymph node metastases as well as on circulating tumor cells (CTCs), the precursors of metastatic disease, has not yet been taken into account. The aim of the present study was to compare the HER2/ER/PR expression profiles of primary tumors, synchronous lymph node metastases and circulating tumor cells in early breast cancer patients and relate the results to 5-year overall (OS) and disease-free survival (DFS).

Patients and Methods: 76 patients with early breast cancer diagnosed between 2006 and 2010 were enrolled in this study. Blood was obtained at the time of first diagnosis of disease and analyzed for CTCs using the *AdnaTest BreastCancer* (Qiagen Hannover GmbH) for the expression of EpCAM, MUC-1, HER2, ER and PR respectively.

Formalin-fixed and paraffin-embedded archival tissues of the primary tumors and the lymph node metastases were analyzed by two pathologists. ER, PR and HER2 expression was assessed by fully-automated immunohistochemistry (Ventana medical Systems, Tucson, AZ, USA) and HER2/CEN17 dual chromogenic in situ hybridization (Zytomed Systems, Berlin, Germany) according to modified ASCO/CAP guidelines (2010 and 2013, respectively).

Results: The detection rate for CTCs was 22% (17/76 patients). In the primary tumors, the expression rate of HER2 was 16% (13/76 patients), 78% for ER (61/76 patients) and 75% for PR (57/76 patients) respectively. Changes in biomarker profiles between primary tumors, metastases and CTCs, as a whole, were observed in 89% of the cases (68/76). The discordance rates between primary tumors and lymph node metastases were 10% for HER2 ($p < 0.001$), 5% for ER ($p = p < 0.001$) and 11% for PR ($p < 0.001$). The intrinsic subtypes between primary tumors and lymph node metastases changed in 16% of all cases (12/76 patients; gain of HER2 in four, activation of HRs in three cases, loss of HER2 in three and loss of HRs in one case becoming triple negative). Of note, both OS and DFS of patients with subtype discordance were reduced to a median of 41 months (mean: 39) vs 56 months (mean: 51).

CTCs were either triple negative or HER2 positive. Discordance rates for HER2, ER and PR status compared to the primary tumors were 16% (12/76 patients), 83% (63/76 patients) and 72% (55/76 patients), respectively. Discordant rates between lymph node metastases and CTCs were 25% for HER2 (19/76 patients), 82% for ER (62/76 patients) and 72% for PR (55/76 patients).

Conclusion: Our preliminary results demonstrate, that changes in molecular profiles are the rule rather than the exception throughout tumour progression in breast cancer. In cases with discordant biomarker profiles, prognosis seems to follow the subtype of lymph node metastasis. Biomarker or subtype shift may be of essential therapeutic significance for individual patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-10

Title: Exome sequencing of circulating tumor cells in metastatic breast cancer

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Body: Aim: We interrogated whether Circulating Tumor Cells (CTCs) can complement metastatic biopsies for genomic analyses.

Patients and Methods: We compared single nucleotide variants (SNVs) and copy number aberrations (CNAs) identified using whole exome sequencing (WES) of DNA from frozen tumor tissue (primary/metastasis), amplified DNA from CTCs and normal DNA from 3 metastatic breast cancer (BC) patients (pts). All samples of the same patient were collected at the same timepoint. CTC isolation was performed using CellSearch and DEPArray systems followed by whole genome amplification (Ampli1 kit). WES was performed using the Illumina HiSeq2000 with 200X targeted coverage. Reads were aligned using bwa. SNVs had to be called by both Haplotype Caller (vs. reference genome) and Strelka (vs. paired normal). CNAs were determined by counting reads in 1MB windows and by comparing tumor/CTC samples with normal DNA. Pairwise concordance of CNAs profiles of different samples from the same patient was assessed using Spearman correlation (ρ). Significance of ρ differences between pts was obtained by Kruskal-Wallis test. Orthogonal validation for selected SNVs was performed.

Results: We studied 3 patients from the 3 major BC subtypes, patient (pt)1 with ER-/HER2+ BC (samples collected at diagnosis, initially metastatic disease), pt2 with triple-negative BC (samples collected 2 years from diagnosis) and pt3 with ER+/HER2- BC (samples collected 8 years from diagnosis).

We first compared tumor tissue and CTCs for SNVs. For pt1, of the 77 SNVs identified in the tumor, 51 were found on at least one of 12 CTCs samples. For pt2, of the 62 SNVs identified in the tumor, 19 were found on at least 1 of 11 CTCs samples. For pt3, of the 225 SNVs identified in the tumor, 48 were found on at least 1 of 3 CTCs samples. Interestingly, by increasing the number of CTCs analyzed, we increased the % of identified SNVs from synchronous tumor tissue. SNVs with high variant allele fraction (VAF) in tumor tissue were detected significantly more often in CTCs: 22% of the SNVs with VAFs <20% were found at least once, compared to 53% and 74% of SNVs with VAFs >20% and >40%, respectively ($p=10^{-12}$, Fisher exact test).

Then, we compared tumor tissue and CTCs for CNAs. As time from diagnosis of metastatic disease to samples collection increased, we observed significantly higher heterogeneity within CTCs from the same patient (median ρ between CTCs was 86% for pt1, 84% for pt2 and 28% for pt3, $p<0.01$) and between CTCs and tumor tissue from the same patient (median ρ was 78% for pt1, 67% for pt2 and 21% for pt3, $p<10^{-4}$). Interestingly, in pt3 one CTC was more similar to the metastasis than the other 2 (ρ of 53%, 21% and 21%). When a phylogenetic tree was constructed for pt3 by combining SNVs and CNAs data, three clones were identified: one clone with an *AKT1* (E17K) and a *TP53* (R248W) mutation and a 8p deletion, a second clone with the above profile plus an 8q amplification and a third clone with an *AKT1* and an *ESR1* (Y537N) mutation and 1p deletion. The metastasis was similar with the first clone.

Conclusions: These data suggest that tumor tissue and single CTC exome sequencing analyses provide complementary information to map tumor heterogeneity. Further validation for potential clinical applications is needed.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-11

Title: Something from nothing? The case for quality control in liquid biopsy studies

Porras TB B, Bains PK K, Ring A, Carrasco S, Forte V, Punj V and Lang JE E. University of Southern California Norris Cancer Center, Los Angeles, CA.

Body: Background: Circulating tumor cells (CTCs) as a liquid biopsy strategy are currently being studied as a surrogate biomarker that may reflect metastatic tumor biology. Given the rarity of CTCs, target enrichment is commonly used to profile the gene signatures of valuable clinical samples to evaluate for multiplexed gene panels of interest. The aim of our study was to evaluate if the NanoString PAM50 could be used for accurate gene expression profiling of CTCs and controls using the research-use-only probeset.

Methods: We collected two 7.5mL EDTA tubes of blood from 12 healthy female volunteers. CTC assays were performed using the ANGLE Parsortix system as a microfluidics filter that separates cells based on size and deformability. The cell lines Hs578T (basal-like) and SK-BR-3 (HER2 amplified) were used to spike 20 cells into n=6 blood tubes per cell line (termed spiked samples). N=12 7.5mL tubes of blood (termed unspiked samples) and n=12 spiked samples were processed using Parsortix for CTC harvesting and lysis using a 10 micron cassette. From each lysate, 5uL was taken for cDNA amplification, multiple target enrichment for 14 cycles, followed by NanoString PAM50 assays. From each of the 12 peripheral blood (PB) samples, we extracted RNA and used 100ng for NanoString PAM50 assays. For cell line controls, 100ng of Hs578T or SK-BR-3 were subjected to NanoString Assays.

Results: Low PAM50 gene expression was observed in all 12 PB samples. Unspiked PB harvested from the CTC assay showed a higher level of PAM50 gene expression compared to PB, suggesting that the target enrichment amplification produces false positive detection of expected breast cancer related transcripts. On ANOVA testing, 10/12 (83%) of unspiked, sorted, target enriched samples had significant differential expression ($p < 0.0001$) of the mean log normalized counts for the PAM50 genes compared to PB.

In spiked experiments using n=20 cells in 7.5mL of PB, sorted Hs578T were found to be triple negative in only 3/6 (50%) while sorted SK-BR-3 were found to be HER2 positive in only 3/6 (50%). On ANOVA testing, the spiked/sorted and bulk were found to have a difference among the mean log normalized counts for the PAM50 genes across all samples for both cell lines ($p < 0.0001$). However, 3/6 (50%) samples had a difference in mean PAM50 gene expression when compared to bulk Hs578t on multiple comparison testing while 2/6 (33%) were statistically significantly different when comparing spiked, sorted SK-BR-3 versus bulk cell line.

Conclusions: Unspiked blood processed via a CTC assay and subjected to target enrichment showed high expression of genes in the NanoString PAM50 assay, likely due to amplification bias. When working with enriched but not ultra-pure CTC samples, amplified gene expression of background leukocytes may influence read counts. This is important to consider in assays that enrich for CTCs but retain a leukocyte background. Further studies will address the effect of the CTC assay procedure and number of leukocytes on accuracy of gene expression of rare CTC mimics. This study emphasizes the importance of selecting genes that are not expressed in PB or performing background subtraction or normalization as strategies for accurate gene expression profiling of CTCs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-12

Title: Prevalence of insulin-like growth factor-1 receptor (IGF1R) expression in circulating tumor cells (CTCs) of patients with early breast cancer and its association with E-cadherin expression and patient survival

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Body: Background: Components of the IGF system are deregulated and IGF1R overexpression is common in breast cancer (BC). E-cadherin (E-CAD) has a prominent role in epithelial differentiation. We investigated the expression of IGF1R in CTCs from patients (pts) with BC and evaluated its correlation with E-CAD expression and survival.

Methods: Cytospins of peripheral blood mononuclear cells (PBMCs), obtained from early (n=62) and metastatic (n=100) pts before adjuvant and 1st-line chemotherapy, respectively, were analysed. Cytospins were double or triple stained with anti-cytokeratin (CK) (A45-B/B3) and anti-IGF1R or with anti- CK, -IGF1R and -E-CAD antibodies, respectively, and evaluated by immunofluorescent microscopy (Ariol system).

Results: CTCs were detected in 20 (32%) and 45 (45%) pts with early and metastatic BC, respectively. IGF1R(+) CTCs were more prominent in early (84% vs 64% of total CTCs, $p=0.0007$) and IGF1R(-) CTCs in metastatic disease (36% vs 16%, $p=0.0001$). Among early pts, 80% had exclusively IGF1R(+) CTCs, 20% had both IGF1R(+) and IGF1R(-) and none had exclusively IGF1R(-) CTCs. The respective values in metastatic pts were 42%, 33% and 25% ($p=0.005$). Early BC pts with exclusively IGF1R(+) CTCs had significantly higher DFS ($p=0.0283$) and OS ($p=0.0016$) compared to those with both subpopulations. Triple IF staining revealed that IGF1R(-)/E-CAD(-) CTCs prevailed in metastatic disease (54% vs 6% of total CTCs, $p=0.0167$). Among early pts, 67% had exclusively IGF1R(+)/E-CAD(+) CTCs, 33% also had IGF1R(-)/E-CAD(-) and none had exclusively IGF1R(-)/E-CAD(-) CTCs. The respective values in metastatic pts were 15%, 77% and 8%, ($p=0.03$). Among CTC(+) early BC pts with detectable CTCs on relapse, the median % of IGF1R(+)/E-CAD(+) CTCs/pt was reduced (59% vs 89%) whereas the median % of IGF1R(-)/E-CAD(-) CTCs/pt increased (41% vs 11%) on disease progression.

Conclusions: IGF1R(+) CTCs are detected more frequently in early compared to metastatic BC. IGF1R and E-CAD are co-expressed at the single CTC-level and the progression from early to metastatic disease is accompanied by a loss of both proteins. These results suggest that IGF1R expression on CTCs contributes to a more differentiated and less invasive phenotype possibly through E-CAD. Accordingly, IGF1R expression in CTCs is associated with improved survival in early BC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-13

Title: A new workflow comprising size based CTC enrichment followed by in situ labeling and micromanipulation with CellCelector™ enables label-free enrichment, isolation and characterization of circulating tumor cells in breast cancer

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Body: Circulating tumor cells (CTCs) are believed to be metastatic precursors of most solid epithelial tumors. Therefore, characterization of their genomic, transcriptomic, and proteomic features may improve cancer diagnosis and prognosis as well as treatment selection and monitoring. CTCs are mostly detected by systems utilizing expression of the epithelial cell adhesion molecule (EpCAM) on CTCs. However, CTCs which have acquired mesenchymal-like features are overlooked although they are believed to be the most malignant cells. Moreover, due to inter cellular heterogeneity single cell isolation by e.g. micromanipulation is required to achieve cellular resolution.

In this project we established a workflow combining the Parsortix™ system to enrich for EpCAM^{low/negative} CTCs by size and deformability and the CellCelector™ micromanipulator to singularize them for further molecular characterizations. First, our workflow was validated by cell-line spiking experiments into blood from healthy subjects (MCF7). Captured cells were stained *in situ* for nuclei, cytokeratin and leukocyte markers, were harvested and then micromanipulated as single cells reaching a capturing rate of 62.66% (\pm 17.79) and a harvesting rate of 72% (\pm 29.61). Then this workflow was applied to a cohort of metastatic breast cancer blood samples which were previously depleted for EpCAM^{high} cells. In 18 out of 22 samples 541 EpCAM^{high} CTCs and in 16 samples 257 EpCAM^{low/negative} potential CTCs were found. Of these 156 EpCAM^{low/negative} and comparable numbers of EpCAM^{high} cells were successfully isolated and lysed for whole genome amplification (Ampli1™). Successful genome-wide amplification was observed in 6.5% of EpCAM^{low/negative} cells vs. 30% of EpCAM^{high} cells indicating lower quality of genomic DNA in the first subpopulation. Sanger sequencing of PCR-amplified exons 9 and 20 of PIK3CA gene revealed heterogeneous mutation status in EpCAM^{low/negative} cells from the same patient. High quality WGA products were also processed for array-based comparative genomic hybridization (aCGH). Comparison of resulting profiles to 267 archived aCGH profiles from breast cancer single cells identified similar somatic copy number alterations: i.e. chromosome 1p loss and 1q gain confirming the tumor origin of captured EpCAM^{low/negative} cells. In addition we could also identify some different aberrations which need to be further investigated.

In summary, our workflow combining size based CTC enrichment followed by in situ labeling and micromanipulation is suitable for single tumor cell analysis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-14

Title: Nuclease-activated oligonucleotide probes for detection of breast cancer circulating tumor cells (CTCs): Early clinical results

Giangrande PH H, Kruspe S, Dickey DD D, Kamboj S, Clark KC C, Urak K, Burghardt E, Smith B, Thomas A and McNamara JO O. University of Iowa, Internal Medicine, Iowa City, IA; University of Iowa, Genetics Program, Iowa City, IA; University of Iowa, MCB Program, Iowa City, IA and University of Iowa, Biostatistics, Iowa City, IA.

Body: Introduction:

A challenge for CTC-based diagnostic tests has been the development of methods with sufficient sensitivity to detect low levels of CTCs. Expense, accuracy and complexity have also limited clinical uptake of CTCs. To overcome these limitations we explored detecting CTCs by measuring their nuclease activity with *nuclease-activated probes*. We present the development of a rapid and highly-sensitive CTC detection assay based on probes that are selectively digested (activated) by target nucleases expressed in breast cancer cells.

Methods:

Nuclease activity in samples from women with Stage IV breast cancer and healthy donors was determined and correlated with clinical data. Patients seen at University of Iowa Clinics were eligible for this IRB-approved study. Blood samples were processed using microfilter (ScreenCell) units for CTC enrichment and converted into cell lysates that were examined by means of three different chemically-optimized oligonucleotide probes. CTC-derived nuclease activity was quantified using a fluorometer. The presence of CTCs was confirmed using established CTC detection methods (e.g. RT-PCR, immunohistostaining).

Results:

Sensitivity of the probe assay was 5 cancer cells in buffer solution and ~200 cancer cells in 1 mL of healthy donor blood. The final study cohort included 28 breast cancer patients and 10 healthy donors. The averaged signal intensities from patient samples were significantly higher compared to the healthy donor control group, presumably arising from CTCs in the blood. Statistical analysis further revealed short incubations in the assay (<20 min) to be optimal. From an ROC analysis we obtained AUC values of 0.8821, 0.8103 and 0.9356 for the three different probes. The oligonucleotide probe being the best predictor of disease yielded 100% sensitivity in the patient samples with a specificity of 70%. The **dsDNA 20 minute probe** was correlated negatively with tumors being ER+/PR+ ($p=0.03$). The **2'f-RNA 0 minute probe** correlated significantly with HER2- tumors ($p=0.04$). In this smaller series other trends were also suggested.

Conclusion:

We describe a novel diagnostic for the detection of CTCs that could overcome limitations of CTC detection assays and could provide a robust diagnostic tool for breast cancer. Future clinical assays derived from this technology could require minimal training and infrastructure and might be developed into a point-of-care testing format.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-15

Title: DETECT V - Detection of the expression of estrogen receptor and human epidermal growth factor receptor 2 on circulating tumor cells from metastatic breast cancer patients

Fehm T, Meier-Stiegen F, Riethdorf S, Schramm A, Polasik A, Niederacher D, Rack B, Taran F-A, Müller V, Janni W and Huober J. Gynecology and Obstetrics, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany; University Hospital Hamburg-Eppendorf, Hamburg, Germany; Gynecology and Obstetrics, University Hospital Ulm, Ulm, Germany; Gynecology and Obstetrics, Klinikum der Ludwig-Maximilians-Universität, Munich, Germany; Gynecology and Obstetrics, University Hospital Tübingen, Tübingen, Germany and Gynecology and Obstetrics, University Hospital Hamburg-Eppendorf, Hamburg, Germany.

Body: Background: The prognostic relevance of circulating tumor cells (CTCs) in metastatic breast cancer (MBC) has constantly been shown in several clinical studies. Nevertheless, the predictive value of CTCs still remains unclear. The DETECT study concept evaluates the impact of CTCs on therapeutic decisions. Patients with hormone-receptor positive, HER2-positive MBC participating in DETECT V are randomized to a dual HER2 targeted therapy (Trastuzumab and Pertuzumab) combined with either endocrine therapy or chemotherapy. Based on the expression of Estrogen Receptor (ER) and Human Epidermal Growth Factor Receptor 2 (HER2) on detected CTCs, an „Endocrine Responsiveness Score (ERS)” is calculated for prediction of endocrine treatment response. Aim of this project was the optimization of staining procedures using immunofluorescence.

Methods: CTCs are enumerated and characterized using CELLSEARCH® CXC Kit which is based on the immunomagnetic enrichment of EPCAM-positive cells and immunofluorescent characterization of these cells. Comparison of staining intensities in different cell lines with known expression of ER and HER2 was used to validate the staining protocol. To validate ER-staining, staining intensities of the following cell lines were compared: SKBR-3 (negative control), MCF-7 (positive control) and T47D (intermediate expression). HER2-staining was validated comparing staining intensities of SKBR3 (positive control), MDA-MB 453 (intermediate expression) as well as MCF-7 (negative control).

Results: Staining intensities were divided into the following categories: negative, weak, moderate and strong. Signal intensities using CELLSEARCH® system correlated with the known expression of HER2 and ER in the different cell lines. No expression of ER could be shown in SKBR3 cells, T47D cells showed weak expression of ER. MCF-7 cells showed moderate to strong expression of ER but no expression of HER2. MDA-MB 453 and SKBR3 showed moderate to strong and strong expression of HER2, respectively. Expression of HER2 and ER could be depicted accurately using CELLSEARCH® CXC Kit. Results were validated in a ring test in all participating laboratories. First patient samples are currently analyzed.

Conclusion: Establishment and validation of the ERS by characterizing CTCs within the framework of the DETECT V study pursues the objective of assessing the potential benefit of endocrine therapy. Validation results and first patient results will be shown.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-16

Title: Circulating tumor cells (CTCs) biomarker evaluation from patients with metastatic breast cancer (MBC) utilizing the TargetSelector™ platform

Hamilton EP P, Yardley DA A, Burris III HA A, Shastry M, Huynh L, Bhikha L and Singh VM M. Sarah Cannon Research Institute/Tennessee Oncology, PLLC, Nashville, TN; Sarah Cannon Research Institute, Nashville, TN; Biocept, Inc, San Diego, CA and Tennessee Oncology, PLLC, Nashville, TN.

Body: Background: Circulating blood biomarkers represent the promise of non-invasive, real-time surrogates for tumor tissue-based biomarkers as well as afford monitoring opportunities over the course of therapy as tumors evolve and acquire resistance to treatment. Circulating tumor cells (CTCs) are cells that disseminate from tumors and can be identified in peripheral blood. CellSearch® is an FDA approved methodology for detecting and enriching this rare CTC population. The prognostic value of CTCs has been established, however the potential to characterize biomarkers on CTCs to inform treatment decisions remains an active area of investigation. Among the emerging platforms for detection and characterization of CTCs is the TargetSelector™ system. While CellSearch® is limited to capture and detection of epithelial derived CTCs based on EpCAM and cytokeratin (CK) respectively, Biocept's TargetSelector™ platform utilizes a novel microfluidic system for CTC enrichment based on an antibody capture cocktail that allows for enumeration of CTCs with variable phenotypes (including CK- CTCs). The CTCs are captured in transparent microfluidic chambers and cells can be viewed *in situ* by fluorescence microscopy and analyzed via immunocytochemistry (ICC), fluorescence *in situ* hybridization (FISH) and PCR analyses.

Methods: Sixty-one patients with metastatic breast cancer consented and provided blood for utilization in the TargetSelector™ platform. Based on the molecular analysis of tissue biopsies, 92% of these patients had ER+ breast tumors. Biomarker expression on captured CTCs was determined by ICC for ER and by FISH for HER2. Concordance between these results and biomarker expression on archival tumor tissue from these patients was calculated.

Results: CTCs were detected in 60 of 61 patient blood samples (range 2–4471); 68% had both CK+ and CK- CTCs, and 32% had only CK- CTCs. None had only CK+ CTCs. Of those with CK+ CTCs, concordance for ER expression between the tissue and blood analyses was 85% (35/41). Concordance was much lower for patients with only CK- CTCs (32%, 6/19). Concordance for HER2 amplification in CK+ patients was 93% (38/41), and 68% (13/19) in CK- patients. For this study, the liquid biopsy was obtained over an extended period of time from when solid tumor ER and HER2 assessments were obtained; this latency period may have influenced concordance levels. For HER2, there was a significantly longer time interval between biopsies for non-concordant than for concordant pairs of samples (58.1 ± 19.7 vs. 30.9 ± 4.3 months) regardless of the CK status. No such difference was seen for the ER analysis.

Conclusions: In this exploratory analysis of 61 patients with MBC, we observed a high rate of detectable CTCs as well as CTC concordance for ER (85%) and HER2 amplification (93%) for patients who had CK+ CTCs. Concordance was less if patients only had CK- CTCs. This may be attributable to heterogeneity in the breast cancer phenotypes associated with these CK- CTCs in addition to inherent issues with testing cell surface markers in this population of cells. The variable latency between the between the collection of tissue and blood samples for these analyses may account for the discrepancies observed.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-17

Title: Establishment and preliminary clinical application of breast cancer CTC detection kit based on immune magnetic lipid microsphere/human breast mammaglobin

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Body: Objective

To explore the effect of breast cancer CTC detection kit based on immune magnetic lipid microsphere (IML) separation and human breast mammaglobin (hMAN) identification, we combined epithelial cell adhesion molecule (EpCAM) IML enrichment and immune fluorescence staining of CK/hMAN (IML/hM-BCD) method.

Methods

74 patients with metastatic breast cancer (MBC) were selected to validate the performance of our integrated CTC capture platform by carrying out side-by-side comparisons with the Cellsearch assay using the same sample. In each study, 15 mL blood was divided equally to identify CTC numbers by employing either Cellsearch and our platform.

Results

In MBC patients, the rates of diagnostic positive detection of Cellsearch and IML/hM-BCD method were 50% and 83.4%, respectively. The IML/hM-BCD method presented a greater ability to capture CTCs from MBC patients. A significant difference in the number (1~5) of CTCs captured by IMLM was observed in the images (2-fold more for IMLM vs Cellsearch™, $P < 0.001$). Furthermore, the morphology and footprint sizes of the cells detected by IML/hM-BCD method offer another approach to validating these observations from the perspectives of cell pathology and cytology. The combined information was utilized to delineate CTCs (DAPI+/CK+/CD45-, cell size $> 5 \mu\text{m}$) from WBCs (DAPI+/CK-/CD45+, cell size $< 15 \mu\text{m}$) and cellular debris. Our immunomagnetic lipid microsphere captured significantly different CTC numbers that corresponded to the patients' clinical data.

Conclusions

The panel of IML separation and combined CK/hMAN identification may serve as representative enrichment and biomarkers for CTCs, thus it presents potentially significant value for monitoring early metastasis, therapeutic efficacy and prognosis for the patients with breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-18

Title: A multiplexed immunofluorescence identifies phenotypic heterogeneity of circulating tumor cells in breast cancer

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Body: Introduction: Circulating Tumor Cells (CTCs) have attracted significant attention as a new class of "liquid biopsy", enabling longitudinal and non-invasive disease monitoring to capture an overall snapshot of individual disease. Primary breast tumors are highly heterogeneous due to their genetic instability. Thus, the presence of heterogeneous populations of CTCs is expected. A number of attempts have been made to address CTC's phenotypic heterogeneity using different methods and approaches. For comprehensive understanding of distinct CTC phenotypes, transcriptomic and cell surface marker studies using single cell analysis separated CTCs into different subgroups with epithelial-, mesenchymal-, and stem-like signatures. These studies concluded that the prevalence of stem-like CTCs is associated with poor prognosis. Immunofluorescence is an ideal approach to understand the heterogeneity of 3 - 4 markers in a large number of distinct CTCs; however, such approach does not address the presence of overlapping phenotypic signatures. The collection method of CTCs adds another layer of complexity since the most commonly adopted CTC enumeration strategy relies on affinity-based selection using antibody against EpCAM, which, although abundantly expressed on epithelial cancer cells, automatically eliminates a large fraction of non-epithelial CTCs. To better understand the whole landscape of this heterogeneous disease, the use of marker-independent unbiased methods to collect CTCs is critical to obtain the overall landscape of phenotypic heterogeneity.

Aim: We aimed to investigate the heterogeneity of breast CTCs on a single cell level using size exclusion- based enrichment principal.

Methods: Whole blood was collected from metastatic breast cancer patients under IRB approved protocol. CTCs were isolated on porous membrane (8 μm) under negative pressure (ISET®) from 10 mL of blood. CTCs were fixed on the porous membrane and used for multiplexed immunofluorescence to investigate the expression of epithelial (CK and EPCAM), mesenchymal (Vimentin, Snail and TWIST), and stem-like markers (CD44, ALDH1, and CD133) on CTCs.

Results: CTCs were isolated on ISET filter from all subtypes, Stage IV patients. CTCs were identified based on their morphological and phenotypical characteristics with our CTC criteria of size of nucleus $\geq 16 \mu\text{m}$, triple negative for α -smooth muscle actin, fibroblast associated protein, and CD45. The CTCs were further characterized based on their mesenchymal and stem-like signatures to understand their heterogeneity.

Conclusions: Multiplex immunofluorescence staining of CTCs collected using the size-based enrichment approach enabled us to identify the heterogeneity of CTCs. The phenotypic heterogeneity represents a corner stone for future studies to identify a subset of CTCs that may be associated with breast cancer patient prognosis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-19

Title: Circulating tumor cells in triple-negative breast cancer patients express prostate related genes and show different genetic profiles in US and German patient cohorts

Kasimir-Bauer S, Gao H, Bittner A-K, Plappert L, Feniuk N, Ueno NT T, Cohen L, Valero V, Woodward WA A, Alvarez RH H, Hoffmann O, Kimmig R and Reuben JM M. University Hospital Essen, Essen, Germany; Hematopathology, Houston, TX; Qiagen Hannover GmbH, Langenhagen, Germany; Breast Medical Oncology, Houston, TX; Integrative Medicine, Houston, TX and Radiation Oncology, Houston, TX.

Body: Background: Triple negative breast cancer (TNBC) is known for its aggressive behavior and poor prognosis since treatment options are limited. Specific biomarkers are urgently needed to treat patients (pts) accordingly. In this regard, circulating tumor cells (CTCs) are discussed to be an ideal surrogate marker for individualized treatment options. Using a multi-marker gene panel for the characterization of CTCs, we recently demonstrated that CTCs in TNBC pts and non-TNBC pts showed different genetic profiles including the expression of the androgen receptor (AR). We here compared CTCs of TNBC pts in two different national Institutions in the US (mainly Afro-American women) and Germany (Caucasian women) to identify potentially ethnical differences and to further explore AR expression related similarities of prostate cancer to TNBC.

Methods: 2x5 ml blood of TNBC pts before therapy (n=18 from the Dep. of Gynecol. and Obstetrics, Essen, GER; n=31 from the MDA Cancer Center Houston, US) were analyzed for CTCs applying positive immunomagnetic selection targeting EpCAM, EGFR and HER2 using the AdnaTest EMT-2/Stem Cell Select (QIAGEN Hannover GmbH, Germany). Subsequently, cDNA was pre-amplified for specific genes using TaqMan PreAmp Master Mix according to in house designed assays. Establishment of a 19 gene qPCR panel was performed for the markers PI3K, AKT2, ERCC1, AURKA, HER2, HER3, EGFR, ALK, AR, BRCA1, c-KIT, c-MET, KRT5, mTOR, NOTCH1, PARP1, SRC1, CD45 (leucocyte control) and GAPDH (housekeeping gene) as well as an internal reference. The cutoff was calculated, taking the false positive rate in healthy donors into account, and defined as $Ct(\text{cutoff}) - Ct(\text{sample}) - [Ct(\text{CD45cutoff}) - Ct(\text{CD45sample})]$. Using the AdnaPanel Prostate Cancer (QIAGEN Hannover GmbH, Germany), cDNA obtained from a subset of 14 pts was further analyzed for the expression profile of AR, prostate specific antigen (PSA) as well as prostate specific membrane antigen (PSMA).

Results: The overall CTC-positivity rate (at least one of the markers expressed) was comparable between both sites (GER: 78%; US: 65%). Similar overexpression frequencies were found for AKT2, AR, AURKA, c-KIT, NOTCH1 and SRC at both sites. However, ALK, BRCA1, HER3 and KRT5 were predominantly found in CTCs of German pts while these genes were not or only weakly expressed in CTCs of the US group. In contrast, c-MET, ERCC1, HER2 and PARP were mainly expressed in CTCs of the US group and rarely in the German group. Interestingly, EGFR was not detected in both groups. Analyzing the overexpression of prostate related genes in a subgroup of 14 TNBC pts resulted in the expression of AR in 21%, PSA in 14%, and PSMA in 36% of cases, respectively.

Conclusion: Although we observed similar gene expression profiles in both patient cohorts, clear differences in some gene expression frequencies were detected at both sites. Whether these findings can be related to ethnic differences will be subject of further investigations. Furthermore, we were able to demonstrate that prostate related genes were frequently detectable which so far have not been discussed in the context of breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-01-20

Title: Inertial focusing circulating breast cancer cells based on the novel 3D-printed serpentine microfluidic chips

Yin P, Hu B, Zhu S and Tian J. School of Life Science and Technology, Xidian University, Xi'an, Shaanxi, China.

Body: Background:

In the field of CTCs, single breast cancer cell information is fundamental for precision testing and prognosis. Traditional method to obtain CTCs information based on CellSearch system always get minimum biological information. Microfluidic chip is a promising tool for handling single cell especially metastatic breast cancer cells in the peripheral blood. Microfluidic fabrication based on polydimethylsiloxane (PDMS) by soft lithography is an expensive, involves substantial human labor, being hard to fully automate method. With the development of 3D printing, which provides extremely fast, cost-effective and less labor used tools, it has recently attracted attention as an approach to fabricate microfluidic chips. CTCs and leukocytes continuously focusing, ordering and positioning individually in microfluidic channel is very important for following single cell detection and analysis.

Methods:

Three type of 3D printers has been studied for printing microfluidic chips, including fused deposition modeling (FDM) (Cloud terminal 3D printer Realme 3D Ltd., China), stereolithography (SLA) (Form 1+, Formlabs, United States) and inkjet-based 3D printer (Projet 3500 HD, 3D system, United States).

In order to focusing breast CTCs and leukocytes into a single line, a serpentine microchannel was used with appropriate Reynolds Numbers, Dean Number, aspect ratio and channel length. Specific function and fabrication method of the chips dictate the design of the microfluidic device so that we design appropriate channels which consider below conditions. (1) Feature size of the microchannel should be printed by 3D printers; (2) Structure of the channel should focus CTCs and leukocytes into a single line one by one with a high throughput.

Results:

From our results, the inkjet 3D printing is suitable for fabricating microfluidic chips and the other two are not. The accuracy of FDM 3D printer, with a resolution of 1 mm channel feature size, is not satisfied with printing microfluidic chips and the material used by FDM was not transparency. Commercial SLA devices, with a resolution of 500 mm channel feature size, will be used to printing microfluidic chips one day with the improvement of photo spot controllable. At present, inkjet printing method, which has a resolution of 150 mm channel feature size, can be used as fabricating microfluidic chips with proper channel size.

Serpentine channels can be used to focus two different cells in a single line. According to calculation and simulation results, some proper conditions are present as follows. R_p should be less than 1 and can't be far lower than 1; Dean Number approximate to 10; R_c should be between 1 ~100. should be approximate 0.1. The results also showed that aspect ratio of microchannel was 0.5 and the minimum channel length was 2.5cm calculated by lateral migration velocity.

Conclusions:

3D printing, which holds several significant advantages, can be utilized to fabricating inertial microfluidic chips. Commercial inkjet 3D printing can be used to printing useful inertial microfluidic chips. Serpentine microfluidic channel with sheath buffer flow can focusing breast cancer cells in a single line for detection such as Raman spectroscopy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-01

Title: Circulating tumor DNA analysis to predict relapse and overall survival in early breast cancer – Longer follow-up of a proof-of-principle study

Turner NC C, Garcia-Murillas I, Chopra N, Beaney M, Kilburn L, Cutts R, Osin P, Nerurkar A, Schiavon G, Hrebien S, Bliss J, Dowsett M and Smith I. Royal Marsden Hospital and Institute of Cancer Research, London, United Kingdom and Institute of Cancer Research, London, United Kingdom.

Body: *Background*

In a previous proof-of-principle study we demonstrated that detection of circulating tumour DNA (ctDNA) in the adjuvant setting, after completion of surgery and chemotherapy for early stage breast cancer, was associated with a high risk of early relapse. Here we present longer follow-up of the same series, to define the predictive power of ctDNA analysis for disease free survival, and assess the potential to predict overall survival.

Methods

We recruited a cohort of 55 women presenting with early stage, primary breast cancer, who were all scheduled to receive neo-adjuvant chemotherapy. The primary tumour was sequenced to identify somatic mutations, identifying at least one mutation in 43 patients. Mutations were tracked with digital PCR to identify ctDNA, in plasma samples taken either at a single post-surgical time point (2-6 weeks post-surgery) or with serial plasma samples taken every 6 months in the adjuvant setting.

Results

At a median 31.7 months follow-up, 42% (18/43) patients had relapsed. Detection of ctDNA at the single post-surgical time point was associated with poor disease free survival, HR=13.6 95%CI (4.5, 41.2) $p<0.001$, and overall survival HR=84.7 95%CI (9.8, 730.4) $p<0.001$. All patients with ctDNA detected in a single post-surgical time point relapsed and died in the follow-up period (7/7, 100% specificity), although the single post-surgery time point had modest 39% (7/18) sensitivity for relapse. Detection of ctDNA at any point in serial sampling was associated with poor disease free survival HR=25.7 95%CI (8.3, 79.8) $p<0.001$ and overall survival HR=47.1 95%CI (6.1, 366.1) $p<0.001$. All patients with ctDNA detected in a serial mutation tracking relapsed in the follow-up period (14/14, 100% specificity), with 78% (14/18) sensitivity for relapse. Sensitivity was limited by 3 cases of brain only relapse and one case of solitary ovarian relapse. Detection of ctDNA in serial sampling had a median lead-time of 8.1 months over clinical relapse.

Conclusion

Detection of ctDNA in the adjuvant setting has a high predictive power for future relapse and death from breast cancer. Therapeutic trials are required to determine whether mutation tracking identifies relapse sufficiently early to allow for further adjuvant therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-02

Title: *ESR1* mutations in circulating tumor cell versus circulating cell-free DNA of metastatic breast cancer patients before first-line endocrine therapy and at progression

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Body: *Background*

Mutations in *ESR1*, the gene encoding the estrogen receptor, have been linked to endocrine resistance in metastatic breast cancer (MBC). It is thought that these mutations are selected during endocrine treatment (ET), but direct evidence that these *ESR1* mutations (*mESR1*) emerge during treatment with endocrine agents is scant. We set out to evaluate *mESR1* in circulating tumor cells (CTCs) and matched plasma cell-free DNA (cfDNA) of MBC patients before start of 1st line ET and at progression.

Materials & Methods

CellSearch-enriched CTCs (≥ 5 CTC/7.5 mL) of 37 MBC patients before start of 1st line ET (baseline cohort; BL) and 38 MBC patients who had progressed on any line of ET for metastatic disease (progressive disease cohort; PD) were evaluated. 52% of the PD patients received one line of ET and 48% more lines, of which 92% contained an aromatase inhibitor. In addition, 10 CellSearch-enriched fractions from healthy blood donors (HBDs) and 46 matched plasma samples (7xHBD, 15xBL, 24xPD) were included. DNA was isolated using the AllPrep kit and cfDNA with the QIAamp CNA kit (Qiagen). Hotspot mutations for *ESR1* (D538G, Y537S, Y537C and Y537N) were evaluated with mutation-specific Taqman assays by chip-based digital PCR (QuantStudio 3D). *mESR1* status was assessed after target-specific *ESR1* amplification capturing all 4 mutations, with thresholds for positivity based on the highest variant allele frequencies in HBDs.

Results

Of all the CTC samples in the BL cohort, 1 patient had mutated Y537N copies, while this mutation was not detected in the matched cfDNA. This patient had received adjuvant treatment with tamoxifen. Also none of the other 14 BL cfDNA samples analyzed harbored *mESR1*. Three PD patients (8%) were positive for *mESR1* in their CTCs (2x D538G and 1x Y537S). These D538G variants identified in CTCs were also detected in the corresponding cfDNA of these patients; for the Y537S mutation no matched cfDNA was available. Seven additional *mESR1* carriers were identified in the other 22 matched cfDNA PD samples, resulting in 38% *mESR1* positivity of the PD plasma samples (7x D538G, 1x Y537C and 1x Y537C).

Conclusion

Sensitivity for detecting *mESR1* in CTC fractions (identified in 8% of the PD patients) was lower than for cfDNA samples. Using cfDNA for *mESR1* detection, we found an higher prevalence of *mESR1* variants in samples obtained at progression to ET (38%) compared to baseline (0%). These findings further substantiate the role of *mESR1* in endocrine resistance.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-03

Title: Circulating inflammatory markers, growth factors, and tumor associated antigens in women with early stage breast cancer receiving neoadjuvant metformin

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Body: Background: Numerous clinical studies have reported that diabetic patients receiving metformin exhibit decreased cancer incidence and cancer related mortality. Metformin's mechanism of anti-tumor action has been attributed to both direct effects on cancer cells and systemic changes in insulin metabolism. Indeed, metformin reduces circulating insulin levels, which may be integral to its effectiveness in the breast cancer (BC) setting where hyperinsulinemia is associated with both recurrence and death. While the impact of metformin on blood glucose and insulin is well documented, its effects on other systemic physiologic and inflammatory factors are unknown. We completed a neoadjuvant "window of opportunity" study of metformin in non-diabetic women with BC and performed a series of analyses on plasma samples to assess the impact of metformin on circulating inflammatory markers, growth factors, and tumor associated antigens.

Methods: Non-diabetic women with early stage, untreated BC were given metformin 500 mg tid for ≥ 2 weeks post diagnostic core biopsy until surgery. Fasting blood was collected at diagnosis and surgery to assess circulating markers pre- and post-metformin administration. Plasma was isolated from blood samples and evaluated for CRP, TNF-alpha, IL-6, IL-8, VEGF, EGF, PIGF (placenta growth factor), CA15-3, and SBGP (serum bone Gla protein). Change scores (post-metformin minus pre-) were calculated and the degree of change characterized by the median change and the rank-biserial correlation. The Wilcoxon signed-rank test was used to test the null hypothesis that the change scores were symmetrically distributed around zero versus more positive or negative change.

Results: A total of 39 patients (mean age 51 years) completed the study and received metformin for a median of 18 days (range 13-40). Metformin was associated with changes in the levels of growth factors, with increases seen in EGF (median increase 1.1 pg/mL, $r=0.42$, $p=0.027$) and VEGF (1.7 pg/mL, $r=0.31$, $p=0.09$). A reduction in PIGF levels (-0.18 pg/mL, $r=-0.6$, $p=0.0028$) was also observed. The tumor associated antigen CA15-3 was significantly reduced after metformin treatment (-0.4 pg/mL, $r=-0.56$, $p=0.0024$) and a marker of bone turnover (SBGP) that has been linked to metastasis was increased (2 nM, $r=0.30$, $p=0.1$). For circulating inflammatory markers, a significant increase in the levels of IL-8 (0.8 pg/mL, $r=0.36$, $p=0.048$) was observed, but changes in TNF-alpha and IL-6 were minimal (TNF-alpha 0.2 pg/mL, $r=0.20$, $p=0.29$; IL-6 0.1 pg/mL, $r=0.14$, $p=0.46$) and no change was seen in CRP (0 mg/L, $r=-0.05$, $p=0.93$).

Conclusions: Short-term metformin administration was associated with alterations in systemic physiologic and inflammatory factors. Such increases in circulating cytokines and growth factors indicate possible alterations in the inflammatory state of the host and/or tumor. Of note, the reduction seen in the tumor antigen CA15-3 may reflect a disease-modifying effect of metformin in BC.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-04

Title: Adipocyte fatty acid binding protein levels predict risk of obesity associated breast cancer

Sauter E, Hao J, Yan X, Kong M and Li B. Hartford Healthcare, Hartford, CT and University of Louisville, Louisville, KY.

Body: Background: Breast cancer risk has been linked to obesity, especially in postmenopausal women. Adipocyte fatty acid binding protein (A-FABP) is found in adipose tissue, and preliminary evidence suggests that its expression in serum is associated with obesity and breast cancer risk. Surgery for morbid obesity appears effective in decreasing morbidity and/or mortality from a variety of diseases of adulthood, including cancer. We evaluated the association of A-FABP expression with 1) body mass index (BMI), 2) breast cancer and 3) change in BMI after obesity surgery.

Hypothesis: Increased A-FABP expression is associated with 1) BMI level, 2) breast cancer, and 3) change in BMI after obesity surgery.

Methods: Serum was collected under an institutional review board approved protocol from two cohorts of obese women: 1) those with or without breast cancer, and 2) those undergoing surgery for morbid obesity. In the first cohort, samples were collected from 101 women prior to surgery to diagnose and treat a concerning breast lesion. In the second, samples were collected from 82 healthy obese women at baseline, and whenever possible, at 3, 6, and 12 months after obesity surgery. A-FABP levels in serum were measured using human A-FABP4 ELISA kits. The student's t-test was used to compare A-FABP levels in the two groups. Linear mixed effect models were used to examine the relationship between A-FABP and BMI or change of BMI over time, controlling for age, menopause status and history of breast cancer.

Results: A-FABP levels were significantly higher in women with cancer than healthy controls among obese women ($p=0.039$), but not among non-obese women ($p > 0.05$). A-FABP levels were higher in obese than non-obese women without ($p=0.038$) and with breast cancer ($p<0.001$). Among healthy obese controls, A-FABP was associated with BMI at baseline ($p=0.016$) and its change over time after surgery ($p=0.005$). The association of A-FABP with BMI was significant for women of normal breast cancer risk regardless of their menopause status ($p=0.012$ for pre-menopause and $p<0.001$ for post-menopause), but not for those with a family history of breast cancer ($p>0.05$). A-FABP was also more predictive of change in BMI in postmenopausal normal risk women ($p<0.001$) than in other groups ($p>0.05$).

Impact: Circulating levels of A-FABP are associated with cancer in obese women, with baseline BMI in healthy women and with change in BMI in normal risk women undergoing surgery for morbid obesity. These findings all point to a clinically important mechanistic role for A-FABP in obesity influenced breast cancer. They also suggest that downregulation of A-FABP may be involved in the decreased risk of cancer, including breast cancer, that has been observed in follow-up after obesity surgery.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-05

Title: Prognostic role of PIK3CA mutational status in circulating tumor DNA (ctDNA) from HER2-positive metastatic breast cancer patients

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Body: Phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) is one of the most commonly mutated genes in breast cancer. But the prognostic and predictive role of PIK3CA mutations remains a matter of debate. It has been suggested that PIK3CA mutations are related to resistance to trastuzumab-based therapies, but they were also related to relatively indolent disease with better progression-free survival. Recently, circulating tumor DNA has been proposed to be a sensitive biomarker for detecting the presence of specific genetic aberrations in various cancer types, providing prognostic and predictive information. In this study, we evaluated prognostic and predictive role of PIK3CA mutations in patients with HER2-positive metastatic breast cancer treated with 1st line trastuzumab and taxane combination chemotherapy. Thirty-four patients with blood samples obtained before 1st line trastuzumab and taxane combination chemotherapy were included in the study. We analyzed two PIK3CA hotspot mutations (E545K, H1047R) in ctDNA by digital droplet PCR (Raindrop™). The samples were collected from April 2005 to December 2011, centrifuged and stored as plasma, and were analyzed in 2015, after 4-10 years of storage period.

Median age was 47 years (range: 31 to 75 years), and histologic type of cancer was ductal carcinoma in all cases. PIK3CA mutations were detected in 21 (61.8%) of 34 patients. Patients with mutated PIK3CA in ctDNA had longer progression-free survival (PIK3CA wild type vs mutant, 11.0 months vs 22.0 months; $P = 0.013$). In hormone receptor positive group, patients with mutated PIK3CA had longer progression-free survival (PIK3CA wild type vs mutant, 12 months vs 18 months, $P=0.17$). Patients with PIK3CA mutations had a lower objective response rate than patients with wild-type, but there was no statistical significance (CR+PR; PIK3CA wild type vs mutant, 84.6% vs 66.7%, $P=0.43$). There was no significant difference in response rate ($P = 0.48$) or PIK3CA mutational status ($P = 0.44$) according to hormonal receptor status (estrogen receptor and/or progesterone receptor). In conclusion, PIK3CA mutations were frequently detected by digital PCR from ctDNA of patients with HER2-positive breast cancer and they were related to significantly better PFS. We plan to analyze the PIK3CA mutation status in matched tumor tissue.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-06

Title: Serial monitoring of circulating tumor DNA in patients with metastatic breast cancer

Patel A, Mukherjee A, Hwang D, Ensor J, Patel TA A, Chang JC C and Rodriguez AA A. Houston Methodist Cancer Center, Houston, TX.

Body: Background: For patients with MBC, there is currently no evidence that changing therapy on the basis of biomarker results improves outcome. Clinical benefit of treatment is defined as complete response, objective response, or stable disease as determined by RECIST criteria on radiological evaluation. Serial measurements of serum biomarkers such as CA2729 and CTCs have proven unsuccessful in predicting clinical benefit. Circulating tumor DNA(ctDNA) has emerged as a potential biomarker that may predict response to therapy or progression of disease. The present retrospective study was conducted to evaluate the relationship between change in ctDNA with clinical benefit determined by clinical and radiological evaluations of patients with MBC patients. **Methods:** We conducted a retrospective, single-institutional study to determine if serial monitoring of ctDNA allele frequency levels predict clinical benefit of a treatment. 55 patients with measurable MBC who had serial monitoring of ctDNA between August 2014 and May 2016 were included. The median age was 55.9 (27–94) years). Clinical outcomes were determined as per standard guidelines. The analysis was performed on all cases that had serial measurements of ctDNA with no change in therapy in between and the repeat blood draw was done within 30 days of repeat radiographic evaluation. The dataset contained 125 observations from 48 unique patients. The relationship between the change in ctDNA and clinical benefit was analyzed using a generalized linear model with a random subject effect to account for the inpatient dependence occurring from obtaining multiple evaluations from the same patient. A logit link function was used akin to logistic regression and a compound symmetric correlation structure was assumed. **Results:** 68.8% of the cases were hormone receptor-positive, 18.8% HER2-positive, and 27.1% TNBC. The treatments received were 58.4% hormonal therapy, 31.2% chemotherapy, 26.4% included anti-HER2 therapy, 2 cases were on targeted therapy, and 1 case was not on any treatment. Three patients had stage 4 disease in complete remission. ctDNA analysis was repeated on average 4 days prior to radiological evaluation. The average time between repeat assessments was 108.5 days. 93% of the patients had a genomic alteration detected at some point during their course of disease. The most common mutations detected were TP53 41.7%, PIK3CA 35.4%, ESR1 18.8%, and ERBB2 amplifications 6.3%. A dichotomized change in ctDNA is a significant predictor of clinical benefit ($p < 0.0001$). The inpatient correlation is estimated to be 0.273 for the transformed variable. The model yields a predicted probability of clinical benefit of 26.9% when the increase in ctDNA is greater than or equal to 0.5 and when the increase in ctDNA is less than 0.5, the a predicted probability of clinical benefit is 78.4%. The concordance of change in ctDNA and change in CA 27-29 was 76.2%. **Conclusions:** Serial evaluation of serum ctDNA may be useful to evaluate molecular response to treatment which may correlate with clinical benefit and potentially guide treatment decisions. Early indication that a chosen therapy is not effective may lead to avoidance of overtreatment and initiation of an alternative regimen. Further, prospective studies are needed.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-07

Title: Delineation of breast cancer based on circulating protein biomarker profile using the OLINK proteomics multiplexed proximity extension assay

Jayachandran G, Cohen EN N, Gao H, Alvarez RH H, Valero V, Lim B, Woodward WA A, Ueno NT T and Reuben JM M. The University of Texas MD Anderson Cancer Center, Houston, TX; Cancer Treatment Centers of America, Newnan, GA; The University of Texas MD Anderson Cancer Center, Houston, TX and The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Expression of cancer related genes and proteins in clinical specimens are the mainstay of personalized targeted therapy. In this study we investigated a blood-based non-invasive and sensitive technique to map biomarkers in breast cancer patients at the protein level instead of expression of cancer related genes. Other multi-platform assays require a large amount of clinical material, multi-step sample processing and complicated data analysis. Proximity Extension Assay performs a harmonious blending of immunoassay and PCR to amplify protein expression signal, thereby enabling multiplexing with small sample input (1 μ l).

Material and methods: Plasma samples (n=25) from patients with inflammatory breast cancer (IBC) or non-IBC, metastatic and non-metastatic, collected prior to starting a new therapy (treatment naive) or a new line of therapy were analyzed using the Proseek Multiplex Oncology I v2 panel (Olink Proteomics, Uppsala, Sweden) for simultaneous detection of 92 human protein biomarkers. In the assay, each protein biomarker is detected by a matched pair of antibodies coupled to unique DNA-tags. Upon binding to the proteins, the correctly hybridized DNA-tags form an amplicon that is measured by a digital PCR. The data was subjected to a dynamic Principal Component Analysis (PCA) with a T-test filter using Multid GenEx software allowing the identification of a specific protein signature with the greatest inter-group differences. Plasma from seven healthy normal donors (HD) was also included in the analysis as comparison.

Results: A plasma protein signature consisting of MK, elf-4B, VIM biomarkers delineated all breast cancers from normal healthy donors. IL-8, CD40L, GDF-15, MCP-1, PARK7, CXCL11, FADD, CAIX, CD69, MIC-A, VEGF-D, EGFR, elf-4B, VIM, and PRSS8 distinguished patients with metastatic breast cancer from normal healthy donors. PCA also identified differences between IBC patients, non-IBC patients, and healthy donors. Clustered association of several protein biomarkers (MCP-1, VIM, HGF, PRSS8 and HER2) distinguished IBC from healthy donors. Plasma from IBC and non-IBC patients differed in their distribution of TNFSF14, CAIX, KN1A, CDHE4 and EGFR. All protein comparisons have p values < 0.05.

Conclusion: These preliminary data suggest that it is possible to distinguish between cancer patients and healthy normal donors, and IBC and non-IBC patients based on a plasma protein profile using the Proseek Multiplex Oncology I v2 panel from OLINK with just 1 μ l of plasma. This pilot study will lead to the establishment of a training set for a protein signature to be applied in a subsequent validation in a larger cohort to assess treatment induced biomarker profile changes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-08

Title: Provista-002: A prospective, multi-center study to determine the effectiveness of a biomarker assay to distinguish benign from invasive breast cancer in women with BI-RADS 3, 4 and 5 imaging reports

Wolf J, Lourenco A, Alpers J, Rohatgi N, Constantini C, Hollingsworth A, Grobmyer S, Pederson H, Haythem A, Polen W, Northfelt D, Morris M, Baker K, Ghosh K, Kass F, Arterbery E, Yang R, Tran Q, Letsios E, Mulpuri R and Reese DE E. Rhode Island Hospital; Avera Cancer Institute; Sutter Institute; Scripps; Provista Diagnostics; Mercy Women's Hospital; Cleveland Clinic; Henry Ford Health System; Summit Medical Group New Jersey; Mayo Clinic; Banner Research Institute; St. Joseph's Hospital; Sansum Clinic; St. Mary's of Michigan and Lahey Clinic.

Body: Introduction:

The ability to detect Breast Cancer (BC), including ductal carcinoma in situ (DCIS) and invasive breast cancer (IBC), in a precise manner remains a challenge. Combinatorial biomarker panels, in conjunction with imaging, were developed to accurately detect BC independently using a serum-based approach. Prior studies demonstrated effectiveness in women under the age of 50; this study tests performance in women ages 25-75. This report presents data on the demographics of enrollment and comprehensive performance characteristics of the biomarker panel models.

Methods:

To rigorously test model performance in women with suspicious breast imaging, we enrolled 1,005 women across 2 cohorts in a national, multi-center, blinded and randomized manner. Serum was collected after a BI-RADS 3, 4 or 5 assessment (as assessed by any imaging modality including mammography, Ultrasound, 3-D Tomosynthesis or MRI) and blood was drawn prior to biopsy and serum frozen for later analysis. Participants who were not diagnosed with BC were followed for 6-12 months.

Serum was evaluated for 11 Serum Protein Biomarkers (SPBs) and 33 Tumor Associated Autoantibodies (TAABs). These measurements were modeled together with patient specific data to determine sensitivity, specificity, PPV and NPV in determining the presence or absence of BC (Grades I through III) in this population.

Results:

As observed in prior studies, the combinatorial use of multiple types of protein biomarkers (SPBs and TAABs) and patient specific data result in superior performance characteristics than each class of biomarker alone. Both cohorts performed similarly where cohort 1 (n= 492) served as a model development set for cohort 2 (n= 502) which served as a validation set. Similar performance characteristics were observed in the validation set which represents the largest rigorously controlled proteomic clinical trial to date.

Conclusions:

These assays were developed to accurately detect BC and reduce false positives in clinically suspicious population. The majority of image-based false positives and false negatives occur in this group. Used properly, this test could ultimately reduce the number of unnecessary biopsies and possibly detect BC at an earlier, more easily treated stage.

Trial Registration:

ClinicalTrials.gov, NCT01839045 and NCT02078570.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-09

Title: Clinical significance of sequential measurements of *ESR1* mutations in plasma cell-free DNA in estrogen receptor positive recurrent metastatic breast cancer patients

Takeshita T, Yamamoto Y, Yamamoto-Ibusuki M, Sueta A, Tomiguchi M and Iwase H. Kumamoto University, Graduate School of Medical Science, Kumamoto, Japan and Kumamoto University Hospital.

Body: Background: The measurement of *ESR1* mutations in plasma cell-free DNA (cfDNA) may transform the management of recurrent metastatic breast cancer (MBC) patients.

We aimed to investigate the clinical significance of sequential measurements of *ESR1* mutations in MBC patients.

Methods: A total of 59 patients (113 plasma samples) with breast carcinoma were enrolled in this study. Cases were selected if archival plasma samples were available from PBC before and after treatment and from MBC gathered more than twice at the time of progression. cfDNA was isolated from the 17 PBC patients (34 plasma samples) and from the 42 MBC patients (99 plasma samples). To investigate any changes in each cfDNA *ESR1* mutation before and after treatment, we analyzed the difference with cfDNA *ESR1* mutations ratio in the first blood sample using droplet digital polymerase chain reaction (ddPCR).

Results:

The median changes in cfDNA *ESR1* mutations ratio in the PBC group tended to be lower than that in the MBC group. The maximum change of each *ESR1* mutation ratio in the PBC groups was used as the minimum cutoff for determining increases in cfDNA *ESR1* mutation ratio. An increase in cfDNA *ESR1* mutations was found in 13 plasma from 12 (28.6%) out of 42 MBC patients. 83.3% (10/12) of MBC patients with increase cfDNA *ESR1* mutations showed a poor response to treatment. Interestingly, 12 MBC patients with increase cfDNA *ESR1* mutations showed various response to endocrine therapy

Patient characteristics of 12 MBC cases with increasing cell-free DNA *ESR1* mutations

Case	Age at 1st blood draw	Site of tissue biopsy	Increasing cfDNA <i>ESR1</i> mutation			After increasing cfDNA <i>ESR1</i> mutation analysis	
			2nd blood draw	3rd blood draw	4th blood draw	ET	BOR to ET
18	66	Lung	Y537S	No rise	No rise	LET	PD
27	31	LN	Y537S	-	-	LHRHa+ANA	PD
44	58	Breast	D538G	No rise	-	Ful	PD
53	40	Ovary	Y537S	-	-	No	-
58	60	Bone	No rise	Y537S	No rise	No	-
62	67	LN	Y537S	-	-	Ful	PD
72	61	Skin	Y537N	-	-	EE2	PR
75	61	Skin	No rise	Y537N	Y537N	Ful /EXE+EVE	PD/SD
77	48	Bone	No rise	Y537S/D538G	-	No	-
89	58	Breast	No rise	Y537N	-	Ful	PD
101	68	Lung	Y537N	-	-	hdTOR	SD
108	56	LN	Y537N	No rise	-	EE2	PR

Abbreviations: ER, estrogen receptor; PgR, progesteron receptor; HER2, human epidermal growth factor receptor 2; PBC, primary breast cancer; MBC, metastatic breast cancer receptor; cfDNA, cell-free DNA; ET, endocrine therapy; BOR, best overall response; LET, letrozole; PD, progressive disease; LN, lymph node; LHRHa, luteinizing hormone releasing hormone agonist; ANA, anastrozole; Ful, fulvestrant; EE2, ethinylestradiol; PR, partial response; EXE+EVE; exemestane+everolimus; SD, stable disease; hdTOR, high dose toremifene.

. In survival analysis, increase cfDNA *ESR1* mutations may predict a shorter duration of post-endocrine-therapy effectiveness ($P = 0.0033$).

Conclusions: We show that sequential measurements of the recurrent *ESR1* mutation in plasma cfDNA of MBC patients is a feasible and useful method of providing relevant predictive information.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-10

Title: Reduced serum B-cell maturation antigen levels predict poor outcome in metastatic breast cancer patients in a phase 3 randomized 2nd-line hormone therapy trial

Ali SM M, Leitzel K, Li M, Udd K, Wang J, Sanchez E, Chen H, Berenson J and Lipton A. Penn State Hershey Medical Center, Hershey, PA; Lebanon VA Medical Center, Lebanon, PA and Institute for Myeloma & Bone Cancer Research, West Hollywood, CA.

Body: Background: B-cell maturation antigen (BCMA) is a member of the tumor necrosis factor receptor family and has two ligands, B-cell activating factor (BAFF) and a proliferation inducing ligand (APRIL). These ligands activate cell proliferation and inhibit apoptosis of normal and malignant B-cells including in multiple myeloma (MM) cell lines. Berenson et al have recently reported that circulating BCMA levels are elevated in B-cell malignancies and can be used to monitor disease and predict PFS and OS for patients with MM, Waldenstrom's macroglobulinemia and chronic lymphocytic leukemia (CLL). On the other hand, recent studies have shown that serum BCMA levels are very low among patients with MM in complete remission with low antibody levels and those with primary immune deficiencies specifically those with combined variable immune deficiency and X-linked agammaglobulinemia. Studies of the potential role of serum BCMA for patients with solid tumors has not been evaluated to date.

Methods: The pretreatment serum from 139 patients with hormone receptor-positive metastatic breast cancer who were enrolled in a phase 3 randomized clinical trial of second-line hormone therapy was evaluated using an ELISA for BCMA. The BCMA ELISA was from R&D Systems (Minneapolis, MN). Serum BCMA was correlated with TTP using categorical serum BCMA cutpoints.

Results: Pretreatment serum BCMA levels had a median of 55.61 ng/ml, an interquartile range of 34.20 and 78.79 ng/ml, and full range from 3.99 to 1193.26 ng/ml. In univariate analysis for TTP, reduced serum BCMA correlated with shorter TTP at the following dichotomous cutpoints: 15 ng/ml [HR=2.60, p=0.064, n=6 (4.3%) of patients below cutpoint]; 20 ng/ml [HR=2.88, p=0.005, n=10 (7.2%) of patients below cutpoint]; 25 ng/ml [HR=2.16, p=0.023, n=13 (9.4%) of patients below cutpoint]; and 30 ng/ml [HR=1.77, p=0.016, n=27 (19.4%) of patients below the cutpoint].

Conclusions: In a phase 3 randomized clinical trial of second-line hormone therapy among patients with hormone receptor-positive metastatic breast cancer, reduced pretreatment serum BCMA was associated with shorter TTP. This may be due to the association of reduced serum BCMA with immune deficiency; and, thus, lead to shorter TTP among patients with metastatic breast cancer. Evaluation of serum BCMA as a new biomarker to predict outcomes for breast cancer and other solid tumor patients deserves further study.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-11

Title: Clinical utility of serial monitoring of circulating tumor DNA (ctDNA) in patients with neoadjuvant chemotherapy (NAC) for locally advanced breast cancer (LABC)

Kim J-Y, Park D, Jung HH, Bae SY, Yu JH, Lee SK, Kim SW, Lee JE, Nam SJ, Ahn JS, Im Y-H and Park YH. Samsung Medical Center, Seoul, Korea; Samsung Genome Institute, Samsung Medical Center, Seoul, Korea; Biomedical Research Institute, Samsung Medical Center, Seoul, Korea; Samsung Medical Center, Seoul, Korea and SAIHST, Sungkyunkwan University, Seoul, Korea.

Body: Introduction: Circulating tumor DNA (ctDNA) is a new biomarker which could guide further treatment. Characterization of tumor mutation profiles is required for informed choice of therapy, given that biological agents target specific pathways and effectiveness may be modulated by specific mutations. It would have clinical utility for neoadjuvant setting also. Thus, we assess the potency of ctDNA to predict tumor response to neoadjuvant chemotherapy (NAC) in locally advanced breast cancer (LABC).

Methods: We performed targeted deep sequencing of 30 plasma DNAs and 10 matched germline DNAs from 10 LABC patients. Serial plasma DNAs were collected at diagnosis, after 1st NAC and curative surgery. For the target enrichment, we designed RNA baits covering a total of ~202kb regions of human genome including a total of 83 cancer-related genes. We constructed the sequencing libraries according to the optimized protocol that we recently reported and sequenced on Illumina HiSeq2500 aiming a mean sequencing depth of ~10,000. After excluding unmapped reads, PCR duplicates and off-target reads, the coverage depths for plasma DNA and germline DNA samples were 2,627x and 4,833x on average, respectively. NAC response was measured by residual cancer burden (RCB) score, calculated as a continuous index combining pathologic measurements of primary tumor and nodal metastases for prediction of distant relapse-free survival.

Results: We analyzed ctDNA and primary tumor tissues from 10 patients with LABC scheduled NAC followed by operation in Samsung Medical Center. Of ten LABCs, one excluded from analysis because of angiosarcoma of breast. Five samples were triple-negative breast cancers (BCs), 2 were HER2 positive BCs and others were ER positive BCs. In tumor response, 1 patient had pathologic complete response (pCR), 1 had RCB class I, 4 and 3 patients did RCB class II and III.

Of 83 genes, in analysis of ctDNA at BC diagnosis, we found 2 to 6 mutations in each samples and 3 mutations were detected averagely. Most common mutation was TP53 (6 patients), followed by PIK3CA mutation. By measuring these mutations in serial ctDNA, we found that ctDNA had disappeared after first cycle of NAC in patient with pCR. In two patients with RCB class I, ctDNA had decreased by more than 10 percent (the level of ctDNA (pg/ml): 455.9 to 30.4, 5.8 to 0.0) of primary plasma sample after first NAC. Two patients increased level of ctDNA had tumor response with RCB class III and one patient had distant tumor recurrence within 3 months after curative surgery. However, correlation between the level of ctDNA and initial stage was not observed.

Patient No.	Initial stage	Surgical stage	RCB score	RCB class	ct DNA at diagnosis (pg/5ml)	ctDNA after 1st NAG (pg/5ml)	Tumor recurrence
1	2A	1	1.333	1	455.9	30.4	No
2	2B	0	0	pCR	446.6	0.0	No
3	3B	2A	1.315	1	5.8	0.0	No
4	2A	1	2.132	2	46.2	55.4	No
5	2B	1	1.797	2	107.8	11.6	No
6	3B	3A	4.090	3	3401.1	5075.5	Yes
7	3A	2B	3.922	3	5088.6	8536.7	No

Conclusions: This preliminary result suggests that serial monitoring of ctDNA would be a potential surrogate marker to predict tumor response and recurrence during NAC in LABC patients. Further results with long-term outcomes are warranted.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-12

Title: Determination of a serum progranulin (GP88/PGRN) level associated with overall survival in metastatic breast cancer patients

Serrero G, Hawkins DM M, Yue B, Hicks D, Tait N and Tkaczuk KR R. A&G Pharmaceutical Inc., Columbia, MD; University of Minnesota, Minneapolis, MN and University of Maryland Greenebaum Comprehensive Cancer Center, Baltimore, MD.

Body: Imaging technologies are the methods of choice in the standard of care (SOC) to monitor therapy response in metastatic breast cancer (MBC) patients. However, such methods are expensive and have limited sensitivity to detect disease response in a timely manner. Measurement of the circulating tumor markers such as CA15-3, CA27.29 and CEA has provided additional minimally invasive methods in disease management of MBC patients. While useful, they have limitations in providing clinicians with a reliable insight into real-time disease monitoring. Understanding of real-time biological processes may provide better biomarkers of the disease state and thus aid real-time clinical management of MBC patients by identifying circulating disease associated biomarkers. Thus, addition of such new circulating biomarkers may improve the management of MBC patients. We have characterized a target biomarker that would fit these criteria, the 88kDa glycoprotein Progranulin (GP88). GP88 is expressed in tumor tissue and not in normal mammary tissue counterpart and is secreted in the circulation of BC patients. Biological studies have established GP88 as one of the critical drivers for breast cancer cell proliferation, survival, invasiveness and drug resistance. Clinical studies have demonstrated that elevated GP88 tumor levels were prognostic for recurrence and that breast cancer patients had a statistically elevated GP88 serum level than healthy individuals. Using tissue and serum tests to detect and quantify GP88 could provide an new strategies for identifying patients at high risk of recurrence and monitoring disease progression in BC patients undergoing therapy. In the present study, we examined whether GP88 serum levels were elevated in MBC patients and whether GP88 serum levels were correlated to patient overall survival.

Under an IRB approved protocol, 92 MBC patients that met the inclusion criteria and were undergoing therapy at the UMGCCC Breast Clinic were consented and enrolled. Clinical and disease characteristics along with serum CA15-3 values were collected as part of the study. Serum samples were collected from each patient during therapy and subsequently the patients were monitored. The serum was stored at -80C until tested for GP88 using a GP88 enzyme linked immunoassay developed in our laboratory.

Statistical analysis using Kaplan-Meier functions established whether there was a correlation between GP88 serum level and overall survival in MBC patients. By analyzing the KM plots at different GP88 cut points, we identified two populations with distinct survival characteristics. When examined more thoroughly the difference in overall survival of patients with <60ng/ml and >60ng/ml was statistically significant ($P=0.0002$). Correlation analysis of serum GP88 and CA15-3 were performed and will be presented. We conclude that circulating levels of GP88 in MBC patients are correlated with overall survival. It would appear that patients that can be managed to have a GP88 below 60ng/ml will survive longer. Thus measuring circulating GP88 levels would provide additional information to that available in today's SOC for monitoring. This valuable insight into real-time disease status will assist clinicians in patient management.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-13

Title: Differential expression of exosomal miRNAs between breast cancer patients with recurrence and no-recurrence

Sueta A, Yamamoto Y, Tomiguchi M, Takeshita T, Ibusuki M and Iwase H. Kumamoto University Graduate School of Medical Science, Japan.

Body: Background

In recent years, there has been a concerted effort to identify biomarkers derived from body fluid in various cancers. Exosomal microRNA (miRNA) has been used as one of useful diagnostic or prognostic biomarkers. We aimed to investigate the prognostic role of the exosomal miRNAs in serum samples derived from patients with primary breast cancer. Additionally, we evaluated whether the exosomal miRNA in serum reflect the origin of primary tumor by means of comparing their expression levels between in serum and tumors.

Patients and Methods

Exosomes in serum sample (500uL) were extracted using ExoQuick (System Biosciences) and miRNAs were isolated using SeraMir™ Exosome RNA Amplification Kit (SBI). We compared the miRNA profile derived from exosome between the patients with breast cancer with recurrence (n=16) and without recurrence (n=16) by miRNA PCR array. Further, we examined the expression of miRNA derived from tumor tissues in the patients with breast cancer recurrence (n=35) and without recurrence (n=39) by qRT-PCR. All samples were collected before treatment and surgery.

Results

Of the 384 miRNAs, 11 miRNAs were significantly expressed; three miRNAs (miR-338-3p, miR-340-5p, and miR-124-3p) were significantly up-regulated and eight (miR-29b-3p, miR-20b-5p, miR-17-5p, miR-130a-3p, miR-18a-5p, miR-195-5p, miR-486-5p, and miR-93a-5p) were significantly down-regulated in the patients with recurrence compared to those without recurrence. Next, we evaluated expression of the above miRNAs in tumor tissues. The patients with recurrence have higher levels of miR-340 at their primary site as well as in the serum. On the contrary, miR-195-5p, miR-17-5p, miR-93-5p, and miR-130a-3p derived from tumor tissues that were down-regulated in the patients with recurrence in serum, were highly expressed in the patients with recurrence than those with no-recurrence. In logistic regression analysis, tumor size, miR-340-5p, miR-17-5p, miR-130a-3p, and miR-93-5p were significantly associated with breast cancer recurrence (each $P < 0.05$).

Conclusions

Several exosomal miRNAs at diagnosis may be useful biomarker to predict the breast cancer recurrence. Moreover, we showed the different expression pattern of miRNAs between tumor tissues and serum. These findings may suggest selective mechanism of release of exosomal miRNA by cancer cells to regulate their progression. Further studies to confirm our results are needed.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-02-14

Title: Circulating cell-free DNA (CFD) measured by a simple fluorescent assay to predict relapse in triple negative breast cancer patients receiving neoadjuvant chemotherapy: A biomarker substudy of prospective observational study (NCT02001519, NCT02001506)

Park K, Woo M, Kim JE, Ahn J-H, Jung KH and Kim S-B. Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea and Medical Oncology and Hematology, Pusan National University Yangsan Hospital, Yangsan, Korea.

Body: Background: Prior technique to measure cell free DNA(CFD) is labor-intensive and expensive, while, recently developed fluorescent CFD assay is more simple and convenient. The aim of this study was to evaluate the role of CFD measured by a fluorescent assay as a biomarker of patients with triple negative breast cancer (TNBC) received neoadjuvant chemotherapy

Methods: We prospectively enrolled patients with TNBC, clinical stage II or III (T>1.5cm or lymph node > 1.5cm), who were scheduled for neoadjuvant chemotherapy. Patients received 4 cycles of adriamycin 60 mg/m² plus cyclophosphamide 600 mg/m² (AC) followed by 4 cycles of cisplatin or docetaxel, and surgery. Plasma samples were obtained from patients before initial chemotherapy (baseline-CFD) and after 4 cycles of AC neoadjuvant chemotherapy (AC-CFD).

Results: This study included 72 patients who met the inclusion criteria. The mean levels of baseline-CFD and AC-CFD were 239±68 ng/mL and 210±66 ng/mL, respectively, and the CFD level was significantly decreased after AC chemotherapy. ($p=0.001$) The baseline-CFD was not associated with initial tumor characteristics. (T stage 1-2 vs. 3, $p=0.313$; N stage 0 vs. 1-3, $p=0.317$) There was no statistically significant difference between patients with response (CR or PR) to AC chemotherapy and those without response in terms of baseline-CFD, AC-CFD, and change of CFD between two values. ($p=0.814$, $p=0.839$, $p=0.927$) With 33.6 months of median follow up, there were 18 cases of relapse. Relapsed group showed numerically higher level of baseline-CFD, although it was not statistically significant. (relapse, 259 ng/mL; non-relapse, 233 ng/mL; $p=0.161$) We performed a ROC curve analysis of baseline-CFD for relapse, and found an area under the curve of 0.62 (95% CI, 0.46-0.78) at 222 ng/mL. Patients with baseline-CFD above 222 showed higher relapses than those below 222. (HR, 2.75; 95% CI, 0.96-7.84; $p = 0.059$)

Conclusions: The baseline-CFD obtained using a simple and convenient fluorescent assay could predict relapse, suggesting baseline-CFD as a potential biomarker for risk stratification of TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-01

Title: An international multicenter study to evaluate reproducibility of automated scoring methods for assessment of Ki67 in breast cancer

Rimm DL L, McShane LM M, Leung SCY CY, Bai Y, Bane AL L, Bartlett JMS MS, Bayani J, Chang MC C, Dean M, Denkert C, Enwere E, Galderisi C, Gholap A, Hugh JC C, Jadhav A, Kornaga E, Laurinavicius A, Levenson R, Lima J, Miller K, Pantanowitz L, Piper T, Ruan J, Srinivasan M, Virk S, Wu Y, Yang H, Hayes DF F, Nielsen TO O and Dowsett M. Yale University School of Medicine, New Haven, CT; Biometric Research Branch, National Cancer Institute, Bethesda, MD; University of British Columbia, Vancouver, BC, Canada; Juravinski Hospital and Cancer Centre, McMaster University, Hamilton, ON, Canada; Transformative Pathology, Ontario Institute for Cancer Research, Toronto, ON, Canada; Mount Sinai Hospital, Toronto, ON, Canada; University of Alberta, Edmonton, AB, Canada; Institut für Pathologie, Charité Campus Mitte, Berlin, Germany; MolecularMD, Portland, OR; Opra Technologies, NeoPro SEZ, BlueRidge, Hinjewadi, India; National Center of Pathology, Vilnius University Hospital Santariskes Clinics, Vilnius, Lithuania; University of California Davis Medical Center, Sacramento, CA; Cancer Diagnostic Quality Assurance Services CIC, Poundbury Cancer Institute, Poundbury, Dorset, United Kingdom; University of Pittsburgh, Pittsburgh, PA; Biomarkers & Companion Diagnostics Group, Edinburgh Cancer Research Centre, Edinburgh, United Kingdom; Queen's University, Kingston, ON, Canada; Breast Oncology Program, University of Michigan Comprehensive Cancer Center, Ann Arbor, MI and Institute of Cancer Research, London, United Kingdom.

Body: Background: The nuclear proliferation biomarker Ki67 has multiple potential roles in breast cancer, including prognosis-based decisions, but unacceptable between-laboratory variability has limited its clinical value. The International Ki67 Working Group (IKWG) has undertaken a systematic program to determine whether Ki67 immunohistochemistry can be analytically validated and standardized across laboratories. Technological advances and broader availability of devices for automated assessment of stained slides raise the possibility that these machines may improve on reproducibility of traditional pathologist-based visual Ki67 assessment.

Aims: To characterize reproducibility of automated machine-measured Ki67 expression using slides previously analyzed in the IKWG phase 3 study that evaluated reproducibility of visual Ki67 assessment.

Methods: Two sets of 30 previously stained slides containing core-cut biopsy sections of breast tumors were circulated to 14 laboratories for scanning and automated assessment of Ki67 expression. Sites were instructed to return average and maximum percentage of tumor cells positive for Ki67 for each slide, where maximum is designed to reflect "hot spot" analysis. Two laboratories returned scores from 2 operators; not all laboratories reported values for maximum Ki67 scores. Different operators were treated as distinct laboratories in analyses. Sixteen and 10 score sets were available for average and maximum Ki67 analyses, respectively, encompassing 7 unique scanner and 10 software platforms. Pre-specified analyses included evaluation of reproducibility across all laboratories as well as within a subgroup limited to those using Aperio scanners. The primary reproducibility metric was intraclass correlation coefficient between laboratories (ICC), regardless of device platform or software.

Results: Geometric means across 30 cases for 16 operators ranged from 11.06% to 38.11% with overall mean 16.75% (95% CI: 14.45-19.42) for average scores. Geometric means for 10 operators ranged from 16.44% to 68.73% with overall mean 25.16% (95% CI: 18.71-33.84) for maximum scores. ICC for automated average scores across 16 operators was 0.83 (95% CI: 0.73-0.91) and ICC for maximum scores across 10 operators was 0.63 (95% CI: 0.44-0.80) although one outlier lab dramatically affected results. For the laboratories using the Aperio platform (8 score sets), ICC for automated average scores was 0.89 (95% CI: 0.81-0.96). These results are similar to ICC of 0.87 (95% CI: 0.81-0.93) reported using these same slides in the Phase 3 visual assessment reproducibility study in which observers counted 500 cells per slide (Leung et al, NPJBrCancer, in press).

Conclusions: Between-laboratory reproducibility for automated machine assessment of average Ki67 is similar to that for pathologist-based visual assessment of Ki67. However, the observed ICC was markedly numerically lower for the maximum score method compared to the average method, suggesting that the maximum score may not be useful as a reproducible measure of proliferation. Automated average scoring methods show promise for standardization of Ki67 scoring, supporting future studies to clinically validate Ki67.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-02

Title: Intratumoral heterogeneity of Ki67 expression in early breast cancers exceeds variability between individual tumours

Focke CM M, Decker T and van Diest PJ J. Dietrich Bonhoeffer Medical Center, Neubrandenburg, Germany and University Medical Center Utrecht, Utrecht, Netherlands.

Body: Background

Regional differences in proliferative activity are commonly seen within breast cancers, but little is known on the degree of intratumoral heterogeneity of Ki67 expression. Our aim was to study intratumoral heterogeneity of Ki67 expression in early breast cancers and its association with clinicopathological features like estrogen receptor (ER) status, grade and histological type.

Methods

The Ki67-labelling index (Ki67-LI) was assessed in hot, cold and intermediate spots of 233 invasive breast cancers by counting a total of 1020 cells, according to a protocol of the International Ki67 in Breast Cancer Working Group. Differences between the spots per tumor were further analyzed for clinicopathological subgroups defined by ER status, grade and histological type.

Results

All clinicopathological subgroups showed significant differences in Ki67-LI between hot, intermediate and cold spots ($p < 0.001$). The coefficient of variance (CV) between the spots was higher in ER positive than in ER negative cancers (72.6% vs 49.2%, $p < 0.001$), and was highest in grade 3 (96.12%), grade 1 (87.27%) and invasive lobular tumors (83.59%), and lowest in medullary (26.48%) cancers. Nested analysis of variance indicated that in both ER positive and ER negative cancers, variance in Ki67-LI within tumours contributed more to the total variance (56% for ER positive, 60% for ER negative cancers) than the variance between tumors.

Conclusion

Intratumoral heterogeneity in Ki67-LI is an ubiquitous phenomenon across various pathological subgroups of breast cancer that may impact assessment of Ki67 levels for clinical decision making, and sheds new light on recommended cut-offs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-03

Title: High concordance of ER, PR, HER2 and Ki67 by central IHC and FISH with mRNA measurements by GeneXpert® breast cancer stratifier assay

Wu NC C, Wong W, Ho KE E, Chu VC C, Rizo A, Davonport S, Kelly D, Makar R, Jassem J, Duchnowska R, Biernat W, Radecka B, Fujita T, Klein JL L, Stonecypher M, Ohta S, Juhl H, Weidler JM M, Bates M and Press MF F. Cepheid, Sunnyvale, CA; Keck School of Medicine/University of Southern California, Los Angeles, CA; Geneuity/MPLN, Maryville, TN; Oregon Health & Science University, Portland, OR; Medical University of Gdansk, Gdansk, Poland; Military Institute of Medicine, Warsaw, Poland; Oncology Center, Opole, Poland; Tokyo Medical University, Ibaraki, Japan; Indivumed GmbH, Humburg, Germany and Josai University, Sakado, Japan.

Body: Current methods for the assessment of ER, PR, Her2, and Ki67 using FFPE tissues are hard to standardize and difficult to perform in Low and Middle Income Countries (LMIC). The GeneXpert® breast cancer stratifier assay (RUO) (BC Strat) is a cartridge-based, RT-qPCR assay of *ESR1*, *PGR*, *ERBB2*, and *MKi67* mRNAs using FFPE specimens. The assay is fast (<2 hours, including <10 minutes of hands-on time) and easy to perform.

The aims of this study were: 1) to evaluate the concordance of BC Strat using different IHC antibodies and scoring methods in a preliminary dataset (Part I); and 2) to assess concordance between BC Strat and high quality standard methods in an expanded dataset (Part II).

Methods

Part I: IHC Antibody Variability

To assess BC Strat concordance with various IHC antibodies, 155 invasive ductal carcinoma blocks were sourced from 3 sites. Twenty-four adjacent slide sections from each block were prepared and shipped to different labs for BC Strat analysis (Cepheid) or IHC and FISH testing. Table 1 summarizes the IHC antibodies and scoring methods used in each reference lab.

Table 1: IHC antibodies and scoring methods used in reference labs

	Antibody for IHC	lab	IHC scoring
ER	SP1	MPLN	Automated (Aperio)
ER	6F11	Path Inc	Automated (Aperio)
ER	6F11	USC	Manual
PR	IE2	MPLN	Automated (Aperio)
PR	16	Path Inc	Automated (Aperio)
PR	PGR636	USC	Manual
Her2*	4B5	MPLN	Automated (Aperio)
Her2*	HercepTest	USC	Manual
Ki67	30-9	MPLN	Automated (Aperio)
Ki67	MIB1	Path Inc	Automated (Aperio)
Ki67	MIB1	USC	Manual

*HER2 FISH (all with PathVysion kit) was performed at USC

Part II: Concordance Study

522 invasive ductal carcinoma FFPE samples were sourced from 5 sites. All BC Strat analysis was performed at Cepheid and all IHC and FISH was performed in the Press laboratory at USC. Overall percent agreement (OPA), positive percent agreement (PPA), and negative percent agreement (NPA) between BC Strat and IHC were determined.

Results

Part I: IHC Antibody Variability

Table 2 summarizes the OPA for BC Strat analysis and IHC performed with different IHC antibodies and scoring methods. Slightly better concordance for ER and PR was observed between the BC Strat and the IHC methods performed at USC. Discordant IHC results were also observed among the reference labs' standard methodologies.

Table 2: Overall Percent Agreement between IHC and BC Strat

	Reference Lab	OPA with BC Strat
ER	MPLN	92%
ER	Path Inc	96%
ER	USC	98%
PR	MPLN	84%
PR	Path Inc	83%
PR	USC	87%
Her2*	MPLN*	93%
Her2*	USC*	91%
Ki67	MPLN	75%
Ki67	Path Inc	67%
Ki67	USC	81%

*for IHC 2+(equivocal), FISH HER2/CEP17 ratio was examined

Part II: Concordance Study

Of the 522 samples tested, 499 (96%) yielded valid results for both BC Strat and IHC (IHC and FISH for Her2). OPA between BC Strat and IHC was 98% for *ESR1*, 91% for *PGR*, 93% for *ERBB2* (IHC and FISH, 97% for Her2 IHC excluding IHC2+), and 81% for *MKi67*.

Conclusion

BC Strat assay measurements for *ESR1*, *PGR*, *ERBB2* and *MKi67* mRNA expression in FFPE breast tumor tissues are highly concordant with IHC and FISH performed by high quality reference labs. Further investigations using clinical outcomes from independent studies including prospective-retrospective clinical trials are in progress.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-04

Title: Concordance of local immunohistochemistry with TargetPrint microarray based assessment of ER, PR and Her2 and BluePrint molecular subtyping in the Symphony Triple A study

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Body: PURPOSE: A decade ago intrinsic biological breast cancer subtypes have been identified which have proven to be of clinical importance in terms of outcome and response to systemic treatment. The aim of the current study is to assess concordance between breast cancer subtypes determined by local immunohistochemistry (IHC) assessment of estrogen receptor (ER), progesterone receptor (PR) and Her2-receptor status and microarray based molecular subtyping in a subset of ER+ early stage breast cancer patients.

PATIENTS AND METHODS: In this prospective observational multicenter study information on local pathology assessment and BluePrint/TargetPrint results were obtained in ER+ Dutch early stage breast cancer patients in whom a 70-gene profile (MammaPrint) was used as they were enrolled in clinical trial based on the existence of controversy regarding the additional value of adjuvant CT. Local IHC assessment of ER, PR and Her2 status were compared with microarray based assessment (TargetPrint/BluePrint) of these characteristics. Reclassification of ER and PR overexpression was assessed by a McNemars test and by Spearman correlation. Furthermore, concordance between the clinical subtypes based on local pathology (Luminal-type: ER+/PR+/Her2-; Her2-type: Her2+ disease) and molecular subtyping was assessed.

RESULTS: Between January 2013 And December 2015 660 patients, treated in 31 hospitals, were enrolled. In 564 (85%) BluePrint and/or TargetPrint was performed in addition to the 70-GS. The majority of patients had ER+/Her2- disease and TargetPrint reclassified 1% (n = 7) of patients as ER-negative ($r = 0,250$, $p < 0,001$). TargetPrint reclassified 7% (n = 40) and 2% (n = 11) of patients for PR and Her2 status respectively (table 1, $r = 0,580$, $p < 0,001$ for PR

Table 1. Concordance between immunohistochemistry and TargetPrint.

Immunohistochemistry	TargetPrint result (ER, PR and Her2 resp.)		Overall discordance (%)	p-value*
	Positive	Negative		
Estrogenreceptor status				
Positive	557 (99%)	6 (1%)		
Negative	n.a.	n.a.	1%	n.a.
Progesterone receptor status				
Positive	474 (96%)	18 (4%)		
Negative	22 (31%)	49 (69%)	7%	0,636
Her2 receptor status				
Positive	3 (30%)	7 (70%)		
Negative	4 (3%)	546 (97%)	2%	0,549
Equivocal	0 (0%)	3 (1%)		

* P-value represents results of the McNemar test.

). Based on IHC 545 (98%) patients were regarded as luminal-type and the remaining 2% as Her2-type. BluePrint reclassified 2% of the clinical luminal-type patients: 4 (1%) patients were reclassified as basal-type and 3 (0%) patients as Her2-type. Of the clinical Her2-type patients 80% (n=8) was reclassified by BluePrint as molecular luminal-type

Table 2. Concordance between clinical subtyping and molecular subtyping according to BluePrint.

Clinical Subtype	No. pts	BluePrint result		
		Luminal	Basal	Her2
Luminal	545	539 (99%)	4 (1%)	3 (0%)
Her2	10	8 (80%)	0	2 (20%)

Note. Overall discordance 3%.

Conclusion: In the current study we observe a high concordance between microarray-based assessment of ER, PR and Her2 and local pathology in Dutch ER+ early stage breast cancer patients. In the small subset of ER+ patients who are considered candidates for 70 GS use and who have HER2+ tumors by IHC molecular typing of HER2 status is of additional value.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-05

Title: A comprehensive molecular analysis of medullary breast carcinoma: A model of immunomodulatory triple negative breast cancer subtype

Romero P, Denizaut G, Benhamo V, Fuhrmann L, Berger F, Bhalshankar J, Gruel N, MacGrogan G, Popova T, Manié E, Stern M-H, Stoppa-Lyonnet D, Rouzier R, Delattre O, Bieche I and Vincent-Salomon A. Institut Curie, Paris, France and Institut Bergonié, Bordeaux, France.

Body: Medullary breast carcinoma (MBC) is a rare subtype of triple negative breast cancer (TNBC) with specific genomic features within the spectrum of basal-like carcinoma. The frequent association between BRCA constitutive mutation and MBC phenotype has been reported previously. In this study including 19 MBC and 36 non-MMB basal-like carcinoma (BLC), we refine the genomic and transcriptomic knowledge about this entity. Using pan genomic Affymetrix genome-wide human SNP6.0 array, we show that i/ MBC harbour more copy number alterations and losses of heterozygosity than BLC and that ii/ the high frequency of BRCAness genomic trait among MBC. Unsupervised and supervised analysis of GeneChip Uman Genome U133 Plus 2.0 Array transcriptomic generated data confirmed that MBC clearly differ from BLC in terms of gene expression level, with 92 genes overexpressed and 154 genes underexpressed in MBC over BLC. Immune response and inflammatory response are the most differentially represented pathways in MBC over BLC. Pro apoptotic gene *BCLG* is by far the more overexpressed gene in MBC. A validation study conducted with RT-QPCR among 526 breast tumors from all molecular subtype confirmed the specificity of *BCLG* overexpression in MBC, which was confirmed at protein level using immunohistochemistry. Moreover, we show that a vast majority of MBC belong to the immunomodulatory TNBC subtype according to *Lehman et al.* Finally, we confirm the better prognosis of MBC toward BLC. Our observations epitomize the importance of developing DNA repair targeting drugs and immunotherapy based trials in order to improve the outcome of such a specific entity.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-06

Title: Improvement in risk predictive value of Nottingham prognostic index by determining GP88 tumor tissue expression for estrogen receptor positive breast cancer patients

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Body: Background: The Nottingham Prognostic Index (NPI), which includes nodal status, tumor size and histological grade was established to provide predictive value information on post-surgery survival for primary breast cancer patients. Attempts to improve NPI's performance have included addition of other biomarker expression and morphological features such as vascular invasion. In the present study, we investigated whether expression of the autocrine growth and survival factor GP88 (progranulin), known to be overexpressed in breast cancer, whereas it is negative in normal mammary tissue, would improve NPI's predictive value.

Methods: We examined the tumor tissue GP88 expression by immunohistochemistry (IHC) in formalin fixed paraffin embedded tissue sections from 508 cases of estrogen receptor positive (ER⁺) invasive ductal carcinoma (IDC) with known clinical outcomes (disease-free and overall survivals) and with known NPI. GP88 IHC tumor tissue expression was determined using an anti-GP88 antibody (clone 6B3) developed in our laboratory. GP88 expression was scored (0, 1+, 2+, 3+) by two board certified pathologists and classified into two IHC score groups of GP88 < 3+ (0, 1+, 2+) and GP88 = 3+. The correlation between GP88 scoring, NPI and disease-free (DFS) and overall survival (OS) outcomes was then examined by Kaplan Meier analysis, Cox proportional Hazard (CPH) ratio and Pearson's χ^2 test.

Results: Kaplan-Meier survival graphs categorized by NPI scores (< 3.4, 3.4-5.4, and >5.4) and by GP88 expression (< 3+ and 3+) showed that for each NPI subgroup, patients with GP88 IHC score of 3+ had a worse disease-free survival (DFS) and overall survival (OS) than patients within the same NPI subgroup with tumors that had GP88 IHC score < 3+. When adjusted for NPI, high GP88 score was highly significantly associated with recurrence with a hazard ratio of 3.30 (95% CI 2.12 to 5.14).

Conclusions: The data suggest that measuring GP88 tumor tissue expression by IHC at time of diagnosis for breast cancer patients with primary ER⁺ IDC could provide recurrence prediction and survival information complementary to that provided by the determination of NPI alone and thus may be useful for risk management of patients diagnosed with breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-07

Title: High concordance of a closed system, near point of care, RT-qPCR breast cancer assay for HER2 (ERBB2) mRNA compared to both IHC/FISH and quantitative immunofluorescence

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Body: Background

Reliable assessment of HER2 receptor status in breast cancer by either IHC or FISH does not unequivocally define receptor expression, due to their semi-quantitative nature, and as many as 10-15% of cases fall into the ASCO/CAP “equivocal” category. Historically, RNA measurements by PCR, including using several commercially available platforms, have been tested, but have not gained broad acceptance for assessment of HER2. However, RNA measurement, as a continuous value, has potential for use for adjudication of the equivocal category. In the current study, we used a real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) assay (GeneXpert® Breast Cancer Stratifier RUO Assay, Cepheid, Sunnyvale, CA, USA) for ERBB2 (HER2) mRNA on the GeneXpert® (GX) platform (Cepheid), which utilizes a closed-system, single-use cartridge, automated system. The RT-qPCR results from GX were then compared with results from clinical HER2 IHC/FISH assays following ASCO/CAP 2013 HER2 testing guidelines (Wolff et al JCO 2013) and quantitative immunofluorescence (QIF).

Methods

Multiple cores (1mm in diameter) were retrospectively collected from 80 formalin-fixed paraffin-embedded (FFPE) tissue blocks with invasive breast cancer seen by Yale Pathology Labs between 1998 and 2011. Tissue cores were processed as lysates for testing at Yale in the automated GX assay. Briefly, gene-specific reverse transcription was performed, followed by RT-qPCR (TaqMan) and ERBB2 mRNA results were expressed as the difference in cycle threshold values (delta Ct) between the endogenous control transcript (CYFIP1) and the ERBB2 mRNA transcript. Results from IHC and FISH were extracted from the pathology reports for the Yale CLIA lab and QIF for each case was measured as previously described (Carvajal et al, JNCI 2015).

Results

Quality control testing showed that the GX platform shows no case to case cross contamination on material from routine histology practices. Concordance between RT-qPCR and IHC/FISH was 91.25% (sensitivity = 0.87; specificity = 0.94; PPV = 0.89; NPV = 0.92) using a pre-defined delta Ct cut-off ($dCt \geq -1$) for HER2 (+) based on prior concordance studies with HER2 IHC/FISH. Concordance between RT-qPCR and QIF was 99% (sensitivity = 0.97; specificity = 1.0; PPV = 1.0; NPV = 0.98) using $dCt \geq -1$ and the pre-defined cut-point for positivity by QIF.

Conclusions

The GX closed system RT-qPCR assay shows greater than 90% concordance with the ASCO/CAP 2013 HER2 IHC/FISH scoring. Additionally, the GX RT-qPCR assay is highly concordant (99%) with the continuous variable HER2 QIF assay, and may better reflect the true continuum of HER2 receptor status in invasive breast cancer. These initial results suggest that rapid, closed system molecular assays may have future value for the adjudication of the ASCO/CAP HER2 equivocal category. This pilot study did not include ASCO/CAP 2013 “equivocal” cases, but that effort is underway.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-08

Title: Simultaneous analyses of HER2 gene and protein status can more precisely predict pathological complete response (pCR) to neoadjuvant trastuzumab with chemotherapy in primary HER2-positive breast cancer

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Body: Background: HER2-positive breast cancer is characterized by overexpression of HER2 protein and/or amplification of the HER2 gene, assessed by immunohistochemistry (IHC) and *in situ* hybridization (ISH), respectively. According to the 2013 ASCO/CAP guidelines on HER2 testing in breast cancer, both protein and gene status can be classified into three categories: positive, equivocal and negative. Gene-protein assay (GPA) is a newly developed technique, in which IHC and dual color ISH (DISH) are simultaneously performed on a single slide. HER2 GPA slides allow bright-field analyses of both protein and gene expression status of each cell. Breast cancer cells can be classified into nine types based on the combination of protein and gene expression with GPA

table 1. Three by three classification of cancer cells by the HER2 GPA analysis

		HER2 protein		
		3+ (positive)	2+ (equivocal)	0, 1+ (negative)
HER2 gene copy number	≥6 (positive)	A	B	C
	4, 5 (equivocal)	D	E	F
	<4 (negative)	G	H	I

Purpose: We aimed to elucidate the relationship between the results of HER2 GPA analysis and the therapeutic effects of trastuzumab-based treatment.

Methods: Fifty three (53) patients with HER2-positive breast cancer, who underwent neoadjuvant trastuzumab with chemotherapy, were analyzed for HER2 status and clinical outcome. First, HER2 protein and gene status in a pre-therapeutic biopsy material from each patient were separately assessed according to the ASCO/CAP guidelines using HER2 GPA slides. Second, all cancer cells in five representative microscopic images of GPA slides were assessed to determine protein expression and to count the gene copy number at individual cell levels. Finally, we investigated the relationship between the mixture composition of the nine cell types and pathological complete response (pCR) of trastuzumab-treated breast cancer.

Results: The GPA results were concordant with the results of IHC in 98% of samples, and with DISH results in 100%. Two hundred eighty nine (289) cancer cells per patient were assessed on average, ranging from 137 to 490 cells. The mean proportion of type A cancer cells was 64%, ranging from 0% to 98%. Patients who had 50% or more of type A cancer cells achieved pCR in 72% of cases (28/39), while patients with less than 50% of type A cancer cells achieved pCR in 7% of cases (1/14) ($P<0.0001$). The mean proportion of type C cancer cells was 7%, ranging from 0% to 60%. Patients who had 10% or more of type C cancer cells achieved pCR in 10% of cases (1/10), while patients with less than 10% of type C cancer cells achieved pCR in 65% of cases (28/43) ($P=0.0016$).

Conclusion: HER2 GPA analyses can more precisely predict therapeutic effect of trastuzumab-based treatment in primary HER2-positive breast cancer. Our study suggests that the HER2 GPA approach would further improve precision therapy in HER2-positive breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-09

Title: Highly reproducible decentralized gene expression analysis of ESR1, PGR, ERBB2 and MKi67 on an automated, standardized molecular diagnostics platform, GeneXpert

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Body: Background: Accurate assessment of ER, PgR, HER2, and Ki67 status using FFPE samples from patients with breast cancer is critical for appropriate patient management, yet immunohistochemistry (IHC), the most common method of assessing these markers, suffers from inherent variability due to pre-analytical/analytical factors and subjective interpretation by pathologists. Here we describe the GeneXpert (GX) Breast Cancer Stratifier RUO Assay (BC Strat), a real time quantitative PCR assay (RT-qPCR) kit which exhibits robust, highly reproducible mRNA measures of *ESR1*, *PGR*, *ERBB2* (*HER2*) and *MKi67*. The aims of this study were: 1) To assess the impact of variability contributed by pathologist-to-pathologist differences in the selection of the tumor area to be assayed, inter-laboratory assay performance, and macrodissection (MAC) vs. no macrodissection (nonMAC) on decentralized BC Strat results; and 2) to assess preliminary concordance of BC Strat with central IHC and FISH results.

Materials & Methods: The GX BC Strat is a cartridge-based RT-qPCR assay performed on the GeneXpert® Instrument (Cepheid) that automates RNA purification, RT-qPCR amplification and detection of mRNA of target genes (*ESR1*, *PGR*, *ERBB2*, and *MKi67*) and a control gene (*CYFIP1*) after sample preparation. Results are reported as delta cycle threshold (dCt) measurements (*CYFIP1* Ct - target gene Ct) in less than 2 hrs.

Thirty-two invasive ductal carcinoma FFPE blocks were sourced based on varying levels of ER, PgR, HER2, and Ki67 expression and % tumor cell content/tumor area. Adjacent sections from each block were prepared as slides and sent to 3 external GX testing sites and a reference lab. Each site used its own pathologists/technicians to determine the % tumor cell content/tumor area, perform MAC or nonMAC, prepare lysates, and perform GX testing. Reference IHC/FISH was performed by Geneuity/MPLN (Maryville, TN, USA). Site-to-site concordance in GX results for MAC or nonMAC samples using pre-defined assay cutoffs per marker were analyzed, as were % tumor cell content/tumor area assessments between pathologists.

Results: BC Strat testing of 32 FFPE breast cancer samples with MAC demonstrated excellent GX site-to-site concordance in positive/negative status calls for ESR1 (100%), PGR (100%), ERBB2 (97%), and MKi67 (97%). In most cases, MAC vs. nonMAC had minimal impact on final positive/negative calls for GX, resulting in high overall concordance for MAC vs. nonMAC for ESR1 (91%), PGR (99%), ERBB2 (99%), and MKi67 (95%). The assay also demonstrated a strong overall concordance with IHC for ESR1 (97%), PGR (81%), ERBB2 (98%, IHC/FISH), and MKi67 (89%).

Conclusion: Decentralized performance of the GX BC Strat Assay is feasible and minimally affected by differences in tumor area selection and MAC techniques across tumors with a range of sizes, invasive tumor cell contents, and expression levels of ER, PgR, HER2, and Ki67. GX BC Strat dCt results across sites are highly reproducible and show good concordance of results with central lab IHC and *HER2* FISH results. These results suggest standardized, decentralized testing of *ESR1*, *PGR*, *ERBB2* and *MKi67* by the GX BC Strat in local pathology labs is feasible.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-10

Title: HER2 expression in clinical breast cancer samples: A novel detection methodology for HER2 protein quantitation using fluorescent nanoparticles

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Body: Background: The human epidermal growth factor receptor-2 (HER2) is a member of a family of transmembrane tyrosine kinase receptors that play an important role in regulated normal cell growth and differentiation. The over-expression of HER2 in a subset of 15-20% of invasive breast cancers has an important bearing on prognosis, as HER2-positive tumors are associated with an aggressive clinical course and poor outcome. Targeting HER2-overexpression has been shown to be a remarkably effective therapeutic modality; however testing of tumor samples to assess the HER2 status of the patient's breast cancer is required. Clinical assays to assess the HER2 status in patients being considered for targeted therapy include immunohistochemistry (IHC), which detects protein over-expression, or fluorescence in situ hybridization (FISH), which detects gene amplification. Both the IHC and FISH methodologies have limitations. Given that the target of the currently approved drugs is the receptor protein, novel detection systems that could more accurately and quantitatively detect HER2 protein in clinical samples over a broad dynamic range would be advantageous and may be clinically helpful.

Material and Methods: A novel detection technology using streptavidin-coated Phosphor Integrated Dot fluorescent nanoparticles (PID) has been developed that can be visualized by fluorescence microscopy and used for quantitative immunofluorescence detection of protein in clinical samples using computer assisted image analysis. In the current study, PID-nanoparticles were used to analyze HER2 protein expression in breast cancer cell lines and 120 well characterized breast cancer samples. These results have been compared with HER2 IHC and HER2 FISH analysis.

Results: The expression levels of HER2 protein from 8 breast cancer cell lines was evaluated by antibody-binding capacity with FACS analysis. Formalin fixed paraffin embedded cell pellets for these cell lines were prepared and used for quantitative HER2 analysis by PID. The PID score/cell for each of these cell lines showed a strong linear correlation with antibody-binding capacity sites/cell by FACS analysis ($R^2 = 0.94$). For the 120 breast cancer samples, PID score/cell was measured and compared against HER2 IHC membrane intensity measure by image analysis (Aperio) and HER2 FISH results. The HER2 PID score/cell showed a correlation coefficient of $R^2=0.72$ versus the average HER2 copy number per cell by FISH, compared with a correlation coefficient of $R^2=0.41$ for HER2 IHC membrane intensity measured by Aperio. For the HER2/CEP17 ratio, the correlation coefficient for the PID score/cell was $R^2=0.79$ compared with a correlation coefficient of $R^2=0.32$ for the HER2 IHC membrane intensity.

Conclusions: PID-nanoparticles demonstrate great potential for the quantitative measurement of protein of clinical interest in routine clinical samples with morphologic confirmation of the tissue being studied. Further studies looking for PID-score thresholds for HER2 gene amplification and correlations with clinical outcome data are warranted and ongoing.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-11

Title: Analytical validation for the RT-qPCR based multiplex mRNA measurements of ER, PgR, HER2, and Ki67 from FFPE tumor tissue using the GeneXpert breast cancer stratifier assay

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Body: BACKGROUND: Accurate assessment of ER, PgR, HER2 and Ki67 status is crucial for breast cancer therapy and patient management. Immunohistochemistry (IHC) assays have been standard diagnostic tools but they are complex and time-consuming to perform and may not be readily available in decentralized laboratories, particularly in low-to-middle income countries. Molecular diagnostics can be a sensitive and accurate alternative to the traditional IHC, and the GeneXpert Breast Cancer Stratifier assay RUO (*BC Strat*), a single use cartridge-based assay performed on the broadly distributed GeneXpert® Instrument (GX) platform, streamlines a technically demanding RT-qPCR process to provide easy, robust, and reproducible ESR1, PGR, ERBB2, and MKi67 mRNA measurements from a 4 µm thick, formalin-fixed paraffin embedded (FFPE) breast tumor section in less than 2 hours.

METHODS: Analytical validation of the BC Strat assay included studies of Linearity/Dynamic Range, Analytical Sensitivity (Minimum Assay Input), Specificity (Potential Interfering Substances), Carryover Contaminations, and Kit and Specimen Slide Stabilities. Both *in-vitro* RNA transcript (IVT) and/or clinical breast cancer tissues were used as sample input materials. Assay results for each analyte were generated from cycle threshold (Ct) values, and final positive/negative test results for each target were also reported as delta Ct values, where $dCt = Ct [CYFIP1 \text{ Reference}] - Ct [\text{Target}]$, using dCt cutoffs previously derived from a clinical sample cohort.

RESULTS: The BC Strat assay demonstrated ≥ 3 log Linear Dynamic Range covering 5-7 logs sample input for all 4 Target dCts with $R^2 \geq 0.95$ independently. The assay currently requires minimal sample input equivalent to CYFIP1 $Ct \leq 35$ ($Ct = 33.5 \pm 1.5 Ct \text{ SD}$) from 20 replicates of 5-level serial sample dilutions using two independent assay lot materials. It is acceptably robust against non-tumor tissues, DCIS, necrotic and/or hemorrhagic cells, lymphocytes, and genomic DNA contaminants. No carryover contamination from the same GeneXpert module was observed over 20 repeat tests during 9 consecutive days. Current real-time data supports assay stability at 5, 30, 37, 45 and 50°C for at least 3 months with minimal performance impact. Sectioned FFPE breast tumor tissues generated consistent dCt results when stored at 4°C and 30°C for up to 1 month before BC Strat assay testing.

CONCLUSIONS: The analytical validations of the BC Strat assay demonstrate an easy and robust mRNA detection with high sensitivity, specificity, reproducibility, and stability in order to aid medical pathologists and clinicians to more rapidly and objectively determine ESR1, PGR, ERBB2, and MKi67 mRNA status in breast cancer. Although stability studies out to 37 months are ongoing, current data suggest the assay is stable for at least 3 months over a wide range of temperatures. The GeneXpert Breast Cancer Stratifier assay potentially offers a rapid, standardized, and cost-effective solution to streamlining complex molecular diagnostics available for use in local pathology laboratories worldwide.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-12

Title: A simulation study depicting the inconsistency of adjuvant online compared to genomic testing when determining the benefit of chemotherapy

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Body: Background: Prognostic factors have been used for years to determine the benefit of adjuvant chemotherapy in breast cancer. However, reporting of the size and grade of the tumor are affected by interobserver variability in reporting. This can result in a change in the results of adjuvant online and an impact on decision making of chemotherapy. On the contrary, genomic testing such as oncotype Dx is reproducible. The purpose of our study was to assess the effect of pathological discordance on the adjuvant online results on a cohort of patients who also underwent oncotype Dx testing.

Materials and Methods: A total of 143 patients' histologies were included in this study. The results of the Phase III WSG-Plan B trial concerning central vs. local grade discrepancy rates were utilized to randomly change the grade of the tumors. 61 percent of grade 1 cancers were upgraded to grade 2 and 2% upgraded to grade 3. 4 percent of grade 2 cancers were downgraded to grade 1 and 26% were upgraded to grade 3. 1 percent of grade 3 cancers were downgraded to grade 1 and 25% were downgraded to grade 2. Likewise, change was made in the size of the tumor in 20 percent of patients. 8 to 10mm, 18-20, 28-30, and 48-50mm changed to 11, 21, 31 and 51mm respectively. 11-13mm, 21-23, 31-33, and 51-53mm was changed to 10, 20, 30, and 50mm. Ten percent of patients had the ER and Her 2 status changed.

Results: The simulation results showed that when the grade was only altered, the spearman correlation of the predicted 10 year mortality on adjuvant online with the original data was significantly changed from 1 to a result of 0.788. When the changed size was additionally added, the coefficient was 0.836. With the altered ER status, the result was 0.749 and with the Her 2 change, the spearman correlation was minimally changed to 0.742. The scattergrams showed a large number of outliers when the alteration in size was added to the altered grade.

Conclusion: Our simulation study confirms that with minimal changes in the clinical parameters because of the lack of perfect correlation between pathologist's results, there is a significant difference in the 10 year predicted mortality on adjuvant online. This is one step further in understanding the lack of correlation between adjuvant online and oncotype Dx, and the inconsistency of chemotherapy decision making with the sole use of adjuvant online.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-13

Title: Immunohistochemistry cannot be used to detect PD-L1/JAK-2 amplification

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Body: Introduction: We have previously identified a 9p24.1 amplicon targeting PD-L1/JAK2 (PDJ) in subset (26.7%) of triple negative breast cancer (TNBC) patients. The PDJ amplicon is common in newly diagnosed and chemotherapy treated TNBCs and is associated with a worse prognosis. Our goal in this study was to determine whether immunohistochemistry (IHC) evaluation could identify those patients who harbor the PDJ amplicon.

Methods: TNBC patients from 1999 to 2015 whose tumors were flow-sorted and evaluated by array-based comparative genomic hybridization (CGH) were identified; paraffin slides were obtained for IHC staining evaluation of JAK-2, phosphorylated STAT3 (pSTAT3), and PD-L1. Pathologic analysis consisted of scoring the stains for intensity (0-3+) and the relative percent of tumor cells with positive staining; positive score was defined as 3+ for any percent staining. Statistical analysis of IHC staining was performed to determine association with the PDJ amplicon defined by focal 9p24.1 copy number gain with CGH \log_2 ratios of >1.0 for each sorted TNBC sample.

Results: Eleven of 43 TNBC patients evaluable by IHC had the PDJ amplicon. There was no association between PDJ amplification and IHC staining for JAK-2, pSTAT3, or PD-L1 regardless of staining intensity or percentage tumor cells positive. Table 1 describes PDJ amplicon status and positivity for JAK2, pSTAT3 and PD-L1. Of PDJ-positive samples, 64%, stained positive for JAK2, 27% positive for pSTAT3, but 0% for PD-L1 in tumor cells. However, in the PDJ-negative group, 69% still stained positive for JAK2, 19% positive for pSTAT3, and 9% for PD-L1.

Table 1: PDJ amplicon status and IHC staining

	JAK2 (3+) (n;%)	pSTAT3 (3+) (n;%)	PD-L1 (3+) (n;%)
PDJ - (n=32)	22;69%	6;19%	3;9%
PDJ + (n=11)	7;64%	3;27%	0;0%

JAK2: Most PDJ-positive samples (10/11, 91%) showed some JAK-2 staining. Although one had only 1+ staining at 5% of the cells, the majority (7/11, 64%) had staining of 3+ and $\geq 10\%$ of the cells with 2 patients demonstrating 3+ staining of 75% and 90% of the cells. However, those patients without the PDJ amplicon also exhibited JAK2 staining, with 66% (n= 22) of PDJ-negative cases staining strongly for JAK-2 (3+) and $\geq 10\%$ of cells. **pSTAT3:** All but one PDJ-positive case demonstrated some staining (1+ or greater) for pSTAT3; the staining intensity and percent positivity were much less than the JAK-2 staining with only 2 cases (18%) having 3+ staining of $\geq 10\%$. Similar staining was seen in the PDJ-negative cohort; 75% of PDJ-negative patients had some pSTAT3 staining (1+ or greater) and 1 patient (5%) exhibited 3+ staining $\geq 10\%$. **PD-L1:** Finally, the PD-L1 staining was low overall with only 18% of PDJ-positive cases demonstrating some staining for PD-L1. Notably, only one case stained 3+ with 20% positivity while the majority of the samples had a 2+ intensity encompassing 5-50% of the cells.

Conclusions: IHC staining for JAK2, pSTAT3, or PD-L1 was not associated with the presence of the PDJ amplicon. Notably positive IHC staining for JAK2 was observed for both PDJ-positive and PDJ-negative tumor cells, thereby nullifying its application as a screening tool for PDJ amplification. Alternative methods, such as fluorescence in situ hybridization, are needed to identify PDJ-positive patients for further study.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-14

Title: Assessment of stromal characteristics in ductal carcinoma in situ of the breast: An inter-observer variability study

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Body: Aim. Ductal carcinoma in situ (DCIS) is considered to be a non-obligate pre-invasive precursor of invasive ductal carcinoma. We previously showed that DCIS with a predominantly myxoid periductal stromal architecture is associated with an increased risk of both overall and invasive recurrence. The aim of this study is to determine a cut-off for the assessment of myxoid stroma and stromal inflammation in DCIS, based on inter-observer variability. Here, preliminary results of the histopathological analysis of 285 DCIS patients are presented, in which the consistency of assessment of stromal features is compared to the reproducibility of other morphological characteristics.

Methods. Hematoxylin/eosin stained tissue sections of 285 DCIS lesions were retrieved from the archives of the Department of Pathology of Leuven University Hospitals, Leuven, Belgium. The following characteristics were independently scored by two pathologists: nuclear grade, intraductal calcifications, extensive comedonecrosis, DCIS architecture, stromal architecture and stromal inflammation. Nuclear grade was scored as low, intermediate or high grade. Intraductal calcifications were scored as absent or present. Extensive comedonecrosis was defined as eosinophilic necrotic debris in >50% of ductal lumina. DCIS architecture was categorized as non-solid or solid, with a cut-off at 50% of ducts presenting with solid growth. Myxoid stroma was defined as loosely arranged collagen fibers interspersed with an amorphous, slightly basophilic substance. Stromal architecture was classified into 4 categories (0%, 1-33%, 33-66% or >66% myxoid stroma). By applying identical cut-offs, stromal inflammation was subdivided into absent, mild, moderate or extensive periductal inflammation. All features were dichotomized, using different cut-offs. Kappa values were determined to assess inter-observer variability.

Results. Nuclear grade was dichotomized as low grade versus intermediate/high grade (κ 0,500), and as grade low/intermediate versus high grade (κ 0,507). The kappa value for scoring myxoid stromal architecture was highest by dichotomization with a cut-off at 33% (κ 0,566), compared to κ 0,454 and κ 0,501 when using 1% and 66% as a cut-off, respectively. A similar analysis for stromal inflammation revealed that the highest kappa value was obtained by dichotomization as 'absent to mild' versus 'moderate to extensive' inflammation (κ 0,724). Dichotomization with cut-offs of 1% and 66% resulted in lower kappa values of κ 0,564 and κ 0,670, respectively. Scores for extensive comedonecrosis showed substantial agreement (κ 0,604). Scores for solid versus non-solid DCIS architecture (κ 0,507), and presence or absence of calcifications (κ 0,664) showed moderate and substantial agreement, respectively.

Conclusions. Adequate prognostic markers require robustness of assessment, i.e. low inter-observer variability and thus high reproducibility. The dichotomous assessment of stromal features in DCIS resulted in similar or even higher kappa values compared to the dichotomous scoring of other histopathological characteristics. This study demonstrates the robustness of dichotomous assessment of both stromal architecture and stromal inflammation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-15

Title: Clinicopathologic and immunophenotypic characterization of angiomatosis of the breast: A rare vascular entity mistaken for low-grade angiosarcoma

McIntire PJ J, Ginter PS S and Shin SJ J. Weill Cornell Medicine, New York, NY.

Body: Angiomatosis of the breast is a rare, benign vascular lesion often mistaken for low-grade angiosarcoma (LGAS), particularly in core biopsy material. While the histopathologic features of this entity have been described in occasional published reports, it has not been well characterized by immunohistochemistry including proliferation (Ki-67) index. We sought to better characterize this entity, particularly in finding distinguishing features from LGAS. Cases of mammary angiomatosis were identified in our breast pathology consultation files spanning 16 years (2000-2015). All available clinical and pathological material for each case were reviewed. Immunohistochemistry for CD31, D2-40 and Ki-67 was performed on a representative whole tissue section from each case. Eight cases from seven patients were identified for study. For one patient, both the primary and recurrent tumors were evaluated. All patients were female with a mean age of 48 years (range: 19-63 years). All were unilateral (left: 5/8, right: 3/8). Most presented with a palpable abnormality or mass (5/8) while fewer were detected by imaging (3/8). The mean tumor size was 4.1 cm (range: 2-9 cm). All cases showed variable sized ectatic, thin walled vessels lined by flat normochromic endothelium diffusely infiltrating stroma. Where present, lesional vessels infiltrated between terminal duct lobular units (TDLUs) but not into the intralobular stroma of TDLUs. Most cases (6/8) showed a combination of lymphatic-appearing and hemangiomatous appearing vessels, the latter notably lined by thin muscular walls. Of the remaining 2 cases, one case showed only lymphatic-appearing vessels and the other showed only hemangiomatous -appearing vessels. CD31 was diffusely positive in all cases. Lymphatic-appearing vessels were D2-40 positive in all but one case. D2-40 was negative or weak in 5/8 hemangiomatous-appearing vessels. Ki-67 indices were <1% in all but one case (5%). Overt features of malignancy including endothelial cell nucleoli, endothelial tufting, papillary formations, solid/spindle cell foci, blood lakes, mitoses, and necrosis were absent in all cases. Mammary angiomatosis is a rare vascular lesion which typically presents as a palpable mass. Despite its characteristic diffuse and infiltrative growth, angiomatosis does not invade into intralobular stroma, an important distinction from LGAS. They are immunoreactive for CD31 and variably so for D2-40. A low (<1%) Ki-67 index helps to distinguish angiomatosis from LGAS which typically shows a higher (>20%) index.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-03-16

Title: Tumor-infiltrating lymphocyte (TIL) assessment distilled into two binary parameters in triple-negative breast cancer (TNBC)

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Body: Background TILs provide prognostic and potentially predictive value, particularly in TNBC. The 2014 International TILs Working Group guidelines for TIL assessment include multiple parameters including quantification of TILs as a continuous variable, TIL location (stromal versus intratumoral), and grading extent of stromal lymphocytic aggregates. Such multi-parameter assessment is onerous for the practicing pathologist and prone to considerable inter-observer variability and low reproducibility. We sought to identify a simplified method to assess TILs at the microscope while maintaining prognostic value in TNBC. **Design** Quantification of mononuclear TILs was performed on a representative H&E slide from 76 cases of primary invasive TNBC. Percent stromal mononuclear TILs (sTILs) within the entire tumor area were estimated. Tumors were defined as LPBC or non-LPBC using a cutoff of $\geq 50\%$ sTILs. TIL location was recorded as only sTILs or both sTILs and intratumoral TILs (iTILs). Stromal lymphoid aggregates were classified as no aggregates, rare aggregates, well-developed aggregates or aggregates with germinal centers. Statistical analyses were performed using disease-free survival (DFS) (range: 16 to 196 months, mean: 110 months) as a primary endpoint. **Results** Improved DFS was noted for LBPC [.25; 95% confidence interval (CI) .08–.76, $P = .0149$]. When classifying peritumoral lymphoid aggregates, only the presence of well-developed stromal lymphoid aggregates was significantly associated with improved DFS (.11, CI: .01-.94, $P = .0440$). The absence of intratumoral lymphocytes trended toward decreased DFS (2.54, CI: .94-6.89, $P = .0667$). **Conclusion** Our findings suggest that morphologic TIL assessment in TNBC can be simplified by evaluating 2 binary parameters: 1) classifying a given tumor as LPBC and 2) the presence of well-developed stromal lymphoid aggregates to predict a given patient's risk of recurrence. Additional independent studies are necessary to validate our findings.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-04-01

Title: Epigenome-wide association study for breast cancer risk using whole genome and target captured bisulphite sequencing: A pooled case-control study nested in the breakthrough generations study

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Body: Background: The field of epigenetic epidemiology has rapidly advanced and recent work has discovered epigenetic markers of breast cancer risk in white blood cell (WBC) DNA. Using Epigenome-Wide Association Studies (EWAS) on the Illumina 450k methylation array, we and others have shown epigenome-wide hypomethylation (-0.2% , $p < 2.2 \times 10^{-16}$) in incident breast cancer cases compared with controls in several prospective cohorts. We have proposed a mechanism that involves cancer risk exposures, lifetime and environmental events, that alter the epigenome and stably modifies an individual's cancer risk. However, more work is needed to establish the clinical utility of this observation, the underlying causes of this variation and to determine whether the 1.7% of CpG sites targeted by the 450k array are representative of the remaining 98% of the epigenome that has not yet been interrogated. Our overall aim is to identify epigenetic traits within the epigenome that are associated with the risk of developing breast cancer.

Methods: We conducted an EWAS using whole genome bisulphite sequencing (WGBS) of WBC DNA from incident breast cancer cases ($n=548$) compared to matched controls ($n=548$) from a prospective cohort (Breakthrough Generations Study) using a DNA pooling approach. Eight DNA pools were prepared in sequencing libraries and sequenced on the HiSeq2500 at PE100bp reads, resulting in ~ 10 -fold coverage per CpG, per library, across ~ 20 million mappable CpG sites. Each pooled sample was also analysed in triplicate on the Illumina 450k methylation array for validation. We used Agilent target capture bisulphite sequencing (TCBS) for technical validation in a subset of breast cancer cases ($n=48$) and matched controls ($n=48$), individually barcoded and sequenced on the MiSeq at PE150bp, aiming for >1000 fold coverage of 425 kb targeted sequence.

Results: Interrogation of specific genomic regions showed that gene-body methylation averages tended to be hypomethylated in cases, while CpG island averages identified both hypo- and hypermethylation. We have validated the same direction of change in 40/51 CpG islands that were covered by the Illumina 450K methylation array and have developed a target capture panel for validation of 960 gene body regions and 224 CpG island regions that were identified as significantly different between cases and controls (average -11% , $FDR < 5\%$). Analysis of TCBS data is ongoing.

Conclusions: Results indicate that epigenome-wide hypomethylation and methylation in specific sites, particularly gene bodies, measured in pre-diagnostic blood samples may be predictive of breast cancer risk, and may thus be useful as a risk biomarker.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-04-02

Title: Improving immunotherapy response by epigenetic modulation

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Body: The presence of predicted neo-antigens in any given tumor is highly correlated with total somatic mutational burden of the same tumor genome. However, many potential neo-antigens are never transcribed, translated, or presented as antigens, partially because they lie in regions of the genome that are transcriptionally-repressed by cytosine methylation of promoter-CpG islands. Treatment with DNA methyltransferase inhibitors (DNMTi) can hypothetically lead to re-expression of many potential neo-antigens. Furthermore, the presence of neo-antigens have been linked to immunotherapy outcomes in patients. Our hypothesis is that tumors with reduced mutational burden can be maximized for neo-antigen presentation by activating transcription and translation of these sequences through DNMTi treatment. Our preliminary results showed that the DNMTi guadecitabine (SGI-110), a second-generation hypomethylating agent, treatment decreases methyl-cytosine in genomic DNA both *in vitro* and *in vivo*. In addition, SGI-110 treatment enhances MHC-II expression on murine mammary carcinoma MMTV-Neu cells upon IFN- γ stimulation *in vitro* and increases T-cell infiltration *in vivo*. Currently, we are performing whole-exome-sequencing and RNA-sequencing to track somatic mutations from DNA to RNA. We will further track somatic mutations from RNA \rightarrow MHC-presented peptide sequences using MHC immunoprecipitation followed by mass spectroscopy analysis. In addition, we will explore the role of guadecitabine therapeutic priming on response to α PD-L1 immunotherapy. These studies will provide a pre-clinical data to evaluate the potential for combined epigenetic and immune-therapy in a clinical trial for breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-04-03

Title: Cross-talk between RNA methyltransferase and demethylase regulates breast cancer growth and progression

Subbarayalu P, Eedunuri VK K, Timilsina S, Rajamanickam S, Viswanadhapalli S, Abdelfattah N, Onyeagucha BC C, Cui X, Mohammad TA A, Huang TH-M, Huang Y, Chen Y and Rao MK K. Greehey Children's Cancer Research Institute, University of Texas Health Science Center at San Antonio, San Antonio, TX; University of Texas Health Science Center at San Antonio, San Antonio, TX; The University of Texas at San Antonio, San Antonio, TX; University of Texas Health Science Center at San Antonio, San Antonio, TX and University of Texas Health Science Center at San Antonio, San Antonio, TX.

Body: The importance of RNA methylation in biological processes has just begun to emerge. However, our understanding of the role and mechanism by which RNA methylation may play a role in tumorigenesis is unclear. Here, we report that RNA N⁶-adenosine methyltransferase protein METTL14 supports breast cancer growth and progression. We show that METTL14 mediates its pro-tumorigenic function by activating the expression of RNA binding protein HuR and consequently inducing the transcriptional stability of key cell cycle-associated genes, TGF β and its signaling partners as well as HMGA2, VEGF and PDGF that are known to play critical roles in cancer growth and metastasis. Importantly, m⁶A RNA methylation levels of METTL14 target genes were significantly lower in breast cancer patients compared to normal matched controls. The decreased RNA methylation level resulting in increased activity of these target genes in breast cancer patients is regulated by a feedback loop between RNA demethylase ALKBH5 and METTL14. Our results reveal that ALKBH5 regulates the expression of METTL14 and HuR, which in turn transcriptionally stabilizes ALKBH5. Furthermore, we demonstrate that like METTL14, ALKBH5 also supports breast cancer growth and progression. Taken together, these findings suggest that a collaboration between RNA methylation and demethylation machinery may set up a threshold of RNA methylation that ensures the stability of genes that play a vital role in normal cell proliferation; and any perturbation of that threshold may lead to uncontrolled expression/activity of those genes resulting in tumor growth and progression.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-04-04

Title: DNA methylation landscapes of breast cancer progression to brain metastasis: A pre-clinical study

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Body: Due to improvements in overall survival rates, breast cancer brain metastasis (BCBM) is a major life-limiting condition with rising incidence. The molecular mechanisms involved in breast cancer (BC) progression to brain metastasis are still poorly understood. We have demonstrated that DNA methylation, a key epigenetic regulatory mechanism, is involved in BC progression to metastatic disease. Here, we characterized the BCBM DNA methylation landscapes according to their molecular subtypes.

Methods: This study included 22 BCBM specimens from 19 patients (ER+/PgR+/HER2-(n=6), HER2+(n=7), and ER-/PgR-/HER2-(TNBC; n=6)) and primary BC specimens with paired molecular subtypes. After microdissection, we generated genome-wide DNA methylomes using HM450K BeadChips. **Results:** Multidimensional scaling revealed that DNA methylation patterns specifically clustered BCBMs according to their respective molecular subtypes. Additionally, we observed that while ER+/PgR+/HER2- BCBM showed a significant global hypermethylation, HER2+ and TNBC BCBMs presented a significant global hypomethylation compared to the respective primary BC specimens. Hypermethylation on ER+/PgR+/HER2- BCBMs mainly affected CpG islands and was significantly enriched in regions overlapping tumor-related genes, such as *APC2*, *CREB3L1*, and *GLI3*; and a large number of developmental genes, including *HOXA9*, *HOXA10*, *HOXB13*, and *PAX6* (**Table1**). On the other hand, hypomethylation on HER2+ and TNBC BCBMs significantly overlapped with conserved intergenic *cis* regulatory elements. Two significantly affected regions included enhancer elements associated with *NEUROD1*, a neurogenic differentiation factor, and *MYT1L*, a Pan-neural transcription factor associated with neuronal differentiation, suggesting an acquisition of brain-like properties (**Table2**). **Conclusions:** Our study suggests a significant role of DNA methylation reprogramming during BC progression to brain metastasis and describes the existence of molecular subtype-specific DNA methylomes. Altogether, this data offers new insight into the complexity of this clinical complication.

Table1:Hypermethylated CpG sites in ER+/PgR+/HER2- BCBM

Chr	Start	Gene	CpG Context	Diff. Meth	FDR P-val
17	46802888	HOXB13	Island	0.82	4.6E-06
16	56666575	MT1M	Island	0.81	3.7E-10
16	85160569	Intergenic	Shelf	0.77	3.7E-17
7	42277807	GLI3	Island	0.73	8.7E-05
6	7728888	BMP6	Island	0.71	3.1E-05
18	11149470	FAM38B	Island	0.70	3.4E-08
11	46317577	CREB3L1	Shore	0.70	5.3E-17
19	1467979	APC2	Island	0.69	3.0E-05
7	27213984	HOXA10	Island	0.69	7.5E-06
11	31826421	PAX6	Island	0.67	7.4E-06
7	27205381	HOXA9	Island	0.65	1.7E-06
13	96204854	CLDN10	Island	0.64	2.1E-04
2	176956678	HOXD13	Island	0.63	2.3E-04

Table2:Hypomethylated CpG sites in HER2+ and TNBC BCBMs

Chr	Start	Gene	CpG Context	Diff. Meth.	FDR P-val
17	46619555	Intergenic	Shore	-0.58	3.2E-04
2	182543233	NEUROD1	OpenSea	-0.56	6.9E-03
12	132900938	GALNT9	Island	-0.56	2.1E-05
13	91827042	Intergenic	Shore	-0.55	8.7E-05
17	46618614	Intergenic	Shore	-0.55	1.4E-05
2	2119853	MYT1L	OpenSea	-0.53	2.5E-04
1	156623074	BCAN	OpenSea	-0.53	2.1E-03
10	128275008	Intergenic	OpenSea	-0.52	1.6E-04
4	80885981	ANTXR2	Island	-0.51	5.2E-03
7	157280331	Intergenic	Shelf	-0.51	6.9E-03
20	59832924	CDH4	Shelf	-0.51	2.7E-02
4	1407858	Intergenic	Island	-0.51	3.7E-03
5	92925721	NR2F1	Shore	-0.51	3.1E-08

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-04-05

Title: RNA immunoprecipitation reveals lncRNA-protein interactions in basal-like breast cancer

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Body: Interactions between long non-coding RNAs (lncRNAs) and proteins contribute to the epigenetic regulation of gene expression, and defects in such interactions have been implicated in several diseases, including cancer. Basal-like breast cancer accounts for approximately 15% of all breast cancer and is a major clinical problem given its poor survival and lack of responsiveness to targeted breast cancer treatments. DNA methylation is non-random and is much more pronounced in basal-like breast cancer (BLBC) compared to other molecular subtypes of breast cancer, raising the possibility that hypermethylation could be important in the genesis of BLBCs. lncRNAs are aberrantly expressed in multiple subtypes of breast cancer, including BLBC, and are considered to have significant prognostic potential. In order to explore the potential roles of DNA methylation and lncRNAs in BLBC, we investigated lncRNA-protein interactions using bioinformatics and molecular techniques, focusing on proteins that actively perform DNA methylation - the DNA methyltransferase (DNMT) family. Our novel bioinformatic methods revealed that BLBC may be sub-classified according to methylation profile in a way that could predict disease survival. We then identified candidate lncRNAs that are over-expressed in BLBC and statistically predicted to interact with DNMT proteins. Using RNA immunoprecipitation of BLBC cell lines, we have identified potential lncRNA-protein interactions that may be involved in epigenetic remodelling in BLBC. The role of these lncRNAs in epigenetic gene regulation and BLBC is currently being explored.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-04-06

Title: Network integration of epigenomic data: Leveraging the concept of master regulators in ER negative breast cancer

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Body: Background: There has been relatively little advancement in changing the management of women with estrogen receptor (ER) negative breast cancer (BC), mainly due to a dearth of actionable therapeutic targets. Therefore, understanding the underlying biology of such a complex disease is necessary for bringing new therapeutic treatments to light. A key question in cancer genomics is how to distinguish 'driver' or essential alterations, which contribute to tumorigenesis, from functionally neutral or 'passenger' alterations that go along for the ride. The majority of published studies investigating driver genes have focused primarily on genomic mutations which have led to novel study designs (basket trials) where patients with a rare mutation, regardless of tumor histology, are matched to a drug expected to work through the mutated pathway. This dominant focus on mutations has overshadowed consideration of inclusion of epigenetic information. This study illustrates network integration of epigenomic data to prioritize ER negative specific methylated genes as potential epigenetic drivers of aggressive disease.

Methods: Causal Networks are small hierarchical networks of regulators whose activity can be modulated by the expression of downstream target genes to enhance understanding of the effect of upstream master regulators on disease or function. A master regulator is a gene or drug positioned as the central or master hub that has the ability to command or influence downstream events. Causal Network Analysis (CNA) was used to find networks that connect upstream master regulators with a 16 candidate methylation gene signature differentiating ER negative from ER positive BC. The 16 ER-negative specific gene methylation signature (*AHNAK*, *ALPL*, *ANXA2R*, *CCND1*, *CIRBP*, *CPQ*, *DST*, *EGFR*, *ESR1*, *GPRC5B*, *HERC5*, *IL22RA2*, *MITF*, *OBSL1*, *POU3F3*, *RB1CC1*) was identified via our drill-down approach starting from a discovery approach (Illumina 450k BeadChip) followed by expression verification, significant rankings in biological pathways (Ingenuity Pathway Analysis), confirmation by targeted sequencing using Illumina MiSeq, and additional filtering in 450K TCGA data sets.

Results: CNA software identified 4 hierarchical networks and their corresponding master regulatory molecules, diethylstilbestrol, transcription regulator SP1, MSH2, and 15-ketoprotaglandin E2. Diethylstilbestrol and SP1 had direct regulatory influence (depth level 1) to the candidate molecules *ALPL*, *CCND1*, *EGFR*, *ESR1* and *CCND1*, *CIRBP*, *EGFR*, *ESR1*, respectively.

Conclusion: In this study, direct regulatory influence, noted for 5/16 candidate genes indicates additional rationale for further consideration and validation of *ALPL*, *CCND1*, *CIRBP*, *EGFR*, *ESR1* as potential epigenetic driver targets in ER negative BC. As cancer therapies become increasingly more specific and begin to move past cytotoxic agents, determining the molecular features of a tumor that predict response to a given drug has become increasingly essential to match patients with optimal therapy. Currently epigenetic therapy in the form of hypomethylating agents (e.g: decitabine) exhibit clinical efficacy in patients with AML and MDS including those patients not responding to cytotoxic therapy.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P1-04-07

Title: Environmental influence on epigenetic markers in the development of breast cancer

He C, Wu W, Gao H, Nephew K, Li L, Schneider B, Han J and Liu Y. Indiana University, Indianapolis, IN.

Body: Aberrant DNA methylation plays an important role in breast cancer initiation and progression. We hypothesize that environmental risk factors contribute to causal alterations in DNA methylation and that such causal changes have already occurred prospectively in normal breast tissue before tumor occurrence. In order to test this hypothesis, normal breast tissue from healthy women must be used. We therefore conducted an integrative molecular epidemiological study that combined the environmental regulation of DNA methylation in normal breast tissue and the differential DNA methylation profiling in tumor and normal breast tissue. We examined *for the first time* the environmental impact on DNA methylation in normal breast tissue from healthy women and determine the association of these epigenetic changes with breast cancer development. Potentially causal DNA methylation markers identified from our study has the potential to make a major scientific and clinical impact in understanding breast cancer etiology as well as developing novel, more effective prevention and treatment strategies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-04-08

Title: Non-nuclear SUMO dynamics regulate mammary epithelial cell transformation

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Body: Objective: The reversible SUMO-posttranslational modification of protein substrates regulates various cellular processes and consistently is important for normal cell physiology. Disruption of SUMO enzymatic components supports onset of various pathophysiological disorders, including cancer. Our recent study identified a splicing event that differentially modulates expression of 2 SENP7 isoforms. The novel SENP7 variant SENP7S is the predominant SUMO protease in normal mammary epithelia; however onset of precancerous ductal carcinoma in situ (DCIS) reduces SENP7S significantly and stays low in all breast cancer (BCa) subtypes. Inversely, the full-length SENP7L isoform is upregulated in BCa and directly leads to BCa metastasis. Unlike SENP7L, SENP7S isoform contribution to carcinogenesis is unclear. Our objective is to define the biological function of this novel deSUMOylase SENP7S in normal versus cancerous epithelial cells.

Results: Consistently with mRNA levels, protein levels of the 2 SENP7 isoforms are also inversely expressed in human BCa versus normal mammary epithelia. SENP7S is localized in the cytosol of MCF10-2A unlike other SUMO proteases including SENP7L that are predominantly nuclear enzymes. Beta-catenin and a component of the Beta-catenin destruction complex, Axin1 are substrates for SENP7S catalytic activity as in the absence of SENP7S, Beta-catenin and Axin 1 are both SUMOylated. Consistently, SENP7S regulates Beta-catenin signaling pathway. SUMOylated Axin1 loses its interaction with Beta-catenin, allowing the Beta-catenin to escape ubiquitylation and further proteasomal degradation. SUMOylated Beta-catenin translocates to the nucleus and activates multiple target genes that potentiate cell proliferation. Increase in cell proliferation and anchorage dependent growth of non-cancerous MCF10-2A cells was observed with inhibition of SENP7S. Additionally, SENP7S depletion potentiates anchorage independent growth of MCF10-2A with significantly greater number and size of spheroids. In comparison to the control. Loss of SENP7S also potentiates the self-renewal properties of the cells, indicative of mammary epithelial cell transformation.

Conclusion: SENP7S modulates Beta-catenin stability and signaling and consequently is critical for normal mammary epithelial cell physiology. Loss of SENP7S, as observed in DCIS, initiates mammary epithelial cell transformation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-04-09

Title: The missing link: Chemicals, epigenetics and breast cancer

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Body: In spite of its high incidence worldwide, only 10-15% of breast cancers can be attributed to hereditary factors, leaving a substantial proportion with unknown causes. Evidence suggests that 26.8% of new breast cancer cases relate to extrinsic factors, such as lifestyle and environmental exposures. Whilst we have increased our knowledge surrounding factors like alcohol and obesity, little is known in regards to chemicals in the environment (e.g. pesticides) or consumer goods (e.g. plasticisers, such as Bisphenol A, and preservatives). Previous research has proved inconclusive, with effects only observed at concentrations considerably higher than what has been identified in human tissues. Furthermore, we have not identified a mechanistic link for many compounds. It has been suggested that epigenetics could provide insight, being defined as '*the link between the environment and our genome*' and research has identified that environmental chemicals can impact on breast cancer development through the epigenome. However this has mainly been undertaken using unrepresentative chemical concentrations and immortalised cell lines – a model significantly different from the human breast.

The presented research aims to investigate the relationship between low-dose chemical exposures (similar to concentrations found in human tissues) and breast carcinogenesis. In order to represent the human breast as closely as possible, primary mammary epithelial cells (HMECs) were grown in three-dimensional co-cultures on a Matrigel base with fibroblasts (HMF) and endothelial (HMMEC) cells. This system allowed for stromal-epithelial interaction within an *in vitro* environment, a crucial factor that has, so far, not been included within much of the previous research. We then exposed the cultures to environmentally relevant concentrations of Bisphenol A (BPA), ranging from 1 nM to 100 nM. These concentrations promoted morphological changes of epithelial 3D structures (acini), which resemble those seen in the early stages of breast carcinogenesis (i.e. filled lumen, uncontrolled proliferation and deformed acini). Utilising Illumina 450k arrays we observed changes in the epigenetic profile of cells in response to the same levels of BPA. In turn, from RNA-sequencing analysis, we confirmed that these changes result in alterations to the gene expression.

From these preliminary findings, we suggest that BPA is involved in early breast carcinogenesis by altering the epigenome. Increasing our understanding of chemical contributions to cancer development not only provides opportunities for cancer prevention, but also allows for more effective risk model development, aiding in identifying individuals at high risk for screening programmes. We therefore outline future work to investigate the impact of chemical mixtures, not just individual chemicals, within this model to further our understanding of the true impact of chemical exposures on the breast.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-04-10

Title: Using the allele specific copy number profile to predict the poor prognosis breast cancer patients of Taiwanese

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Body: Introduction: Strategies are needed for the identification of a poor response to treatment and determination of appropriate strategies for breast cancer patients. As an integral component in the tumor's genetic profile, knowledge of somatic copy number aberrations can lead to insights into the tumor's genetic history and may allow for more accurate prognosis and most appropriate treatment for the patient. By quantifying the number of copies of each allele at each variant loci rather than the total number of chromosome copies, allele-specific copy number (AsCN) estimation is an important step to the characterization of tumor genomes. In this study, AsCN profile was generated and used to predict the poor prognosis breast cancer patients.

Methods: The blood DNA samples from 56 Taiwanese breast cancer patients were collected from Sep 2005 to May 2015. There are 24 patients were administrated with chemotherapy (42.85%), and 9 patients were recurrent (16.07%), 5 patients were metastasis (8.92%). Affymetrix Human SNP 6.0 microarray chip was utilized to detect the alleles and copy number (CN) variations. The state of CN was distinguished from the intensity of the CN probe on the array. These states of CN were created from unpaired analysis with reference samples (HAPMAP Asian 44 samples), and utilized BRLMM-P+ algorithm from Affymetrix. The AsCN profile of patients were established with HMM algorithm, and analyzed by unpaired analysis in Partek Genomic Suite 6.6 (Partek Inc., St. Louis, MO, USA).

The final result was reported after combined the AsCN result with the segmentation result of copy number variation (CNV) analysis. Two segmentation algorithms were utilized to determine the CNV region. First is circular segmentation algorithm, with minimum 10 genomic markers in each segment and P-value threshold < 0.0001 . Second algorithm is hidden Markov model segmentation (HMM), with the parameter sigma equal to 0.99 and likelihood equal to 0.985.

Results: Four allele-specific imbalance regions which located on gene ABCB11 in 2q31.1, PREX2 in 8q13.2, GPHN in 14q23.3, and mir8062 in 20p 12.3 were detected and highly related to the poor prognosis of chemotherapy. The mean of CN state of these regions was lower than 1.2. The most significant region is the located on gene mir8062 in 20p12.3 (hsa-mir-8062), 22 of 54 patients were detected to bring this imbalance region on genome. The function of this miRNA is not clear now, and needs more investigated.

Conclusions: We have demonstrated the feasibility of finding the association of allele-specific imbalance regions on genome with the poor prognosis in breast cancer by generating the AsCN profile from patients. This preliminary result shows these alleles-specific imbalance regions of the genome have the possibility of playing the important role in the recurrent and metastasis of breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-04-11

Title: The epigenetic landscape of oestrogen receptor-alpha positive breast cancer

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Body: Background

Oestrogen receptor-alpha (ER- α) positive primary breast cancer carries a 30%-40% relapse rate owing to the development of endocrine resistance. Although the mechanisms underlying endocrine resistance in breast cancer remain unclear, recent evidence suggest that epigenetic reprogramming plays a central role. The aim of our study is to study the epigenetic landscape of ER- α positive breast cancer in primary and metastatic breast cancer tissue samples.

Methods

We collected 55 (39 primary + 16 metastatic samples) fresh frozen breast cancer tissue samples which were processed for chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq). Using histone mark H3K27ac we mapped the entire repertoire of regulatory regions. We developed a novel computational approach in analysing this unique dataset in order to account of the intra-sample heterogeneity, allowing us to identify potential molecular signatures responsible for cancer progression.

Results

All samples possessed at least 50% tumour cellularity and all primary samples were confirmed as ER- α positive whilst the metastatic samples were utilised based on a previous background of ER- α positive primary breast cancer. Hormonal status and histological reports were obtained for all tumour samples. Downstream analysis was based on 47 tumour samples which provided 90% and 100% coverage of all active enhancers and promoters, respectively, with no difference in peak size between the two groups. The tumour samples exhibit a high degree of heterogeneity in enhancers, with promoters showing a much higher conservation rate. However, we also identified a cluster of enhancers common to the majority of patients. Using our custom workflow, we de-convoluted heterogeneous and shared enhancers and identified novel transcription factors (TFs) independently enriched in the two cluster of enhancers. Some of this TFs have a significant prognostic value in a meta-analysis of 1466 ER- α positive expression profiles (O.S. $p=0.00016$). Further proof of concept using a highly shared enhancer identified SLC9A3R1 as a potential new oncogene for ER- α positive breast cancer. Finally, we used epigenetic stratification to improve breast cancer diagnosis and prognosis. Overall, our work represents the first systematic annotation of ER- α breast cancer's epigenome. This work should form the basis for future characterization of the epigenetic drivers of breast cancer progression.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-01

Title: Landscape of somatic mutations in inflammatory breast cancer whole-genome sequences

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Body: Goal: Inflammatory breast cancer (IBC) is a rare, aggressive form of breast cancer that is characterized by a highly metastatic phenotype. Numerous previous attempts failed to identify, recurrent, IBC-specific gene expression or DNA copy number alterations. We performed whole genome sequencing (WGS) of IBC biopsies obtained before any therapy to define a comprehensive genomic landscape of this disease.

Methods: Illumina paired-end whole genome sequencing (WGS) of 20 IBC (n=9 ER+, n=11 ER-) and matched normal samples were performed with median coverage of 60X and 40X for cancer and normal, the percentages of mapped reads were 99.3% and 99.2%, respectively. We identified germ-line and somatic variants, indels as well as large scale structural variants, using GATK Haplotype Caller, MuTect and CREST, respectively. We performed the same analysis on WGS data from 23, age, race and ER and HER2 matched, non-IBC (n=12 ER+, n=11 ER-) from the TCGA for comparison. Variants in both coding and noncoding sequences were categorized by FunSeq to identify potential drivers. Mutation clustering in each gene, as well as significantly mutated non-coding regulatory modules, were identified using LARVA. DeconstructSigs were used to decompose the mutational spectrum of each cancer into 30 validated, mutational signatures provided by COSMIC. Contributions of each validated signature to mutations in IBC vs. non-IBC were compared using Welch's t-test.

Results: We identified 118,818 somatic variants in the IBC samples (median: 3,856; minimum: 1,109; maximum: 24,815) including 1,060 variants (~0.9%) in coding regions. 5,287 somatic indels and 5,959 large scale structural variants were detected including 1,028 insertions and 1,857 deletions. Recurrent, non-synonymous mutations were detected in the coding region of *GRIN2A* gene in 3/20 IBC samples (15%), (previously reported as a potential driver mutation in 1.7% of breast cancers). Other significant mutations in coding regions included *GRHL1*, *PIK3R2*, *ESR1*, *FLG2* and etc. Three DNase I hypersensitive sites (DHSs) in non-coding regions were altered in 20% (4/20) IBC samples vs. fewer than 8.7% (2/23) in non-IBC. Mutational frequency of *GATA3* is 80.0% vs. 47.8% (p=0.03), and *PTEN* is 45.0% vs. 73.9% (p=0.05), in IBC vs. non-IBC samples when including both coding and non-coding variants. Contributions of mutational signature 9, that is associated with polymerase η , were significantly higher in IBC cohort than non-IBC cohort (p-value=0.056).

Conclusion: This is the first whole genome sequencing analysis of IBC and comparison with the results from non-IBC. We identified promising candidate drivers in the coding sequence and in non-coding regulatory modules of expressed genes. We also identified mutational signature 9, and mutations in several DHS as significantly more frequent alterations in IBC compared to non-IBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-02

Title: Genomic, transcriptomic and immune features of breast cancer according to the patient's body mass index at diagnosis

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Body: Background: According to the latest estimates, 39% of women worldwide are either overweight or obese. This percentage varies by country with estimates as high as 63% in the USA. High body mass index (BMI) has been recognized as a risk factor for developing breast cancer (BC), especially estrogen receptor (ER)-positive and triple-negative BC in post and premenopausal women, respectively. Additionally, increased BMI has been associated with adverse survival, although several studies reported discrepant results across the different BC molecular subtypes. To the best of our knowledge, no study has so far explored the relation between the BMI of the patient and the genomic, transcriptomic and immune features of the associated tumor.

Material and methods: We collected the BMI at diagnosis for the BC patients included in the whole genome and transcriptome sequencing substudy of the International Cancer Genome Consortium (Nik-Zainal *et al.* Nature 2016). BMI was available for 381/560 of the initially sequenced patients. We investigated the association of BMI with classic clinico-pathological variables, mutational burden, substitution/rearrangement signatures, genomic drivers, transcriptional features and tumor infiltrating lymphocytes (TILs) in all patients and in the subgroups defined by the ER, PgR and HER2 as well as by menopausal status.

Results: The only clinico-pathological variable associated with BMI was the age at diagnosis. Henceforth, all results were further adjusted for age. A higher number of substitutions was associated with increased BMI in ER+/HER2- and HER2+ BC with a low statistical evidence. Specifically, substitutions belonging to signature 1A (C>T substitutions at NpCpG) were significantly associated with BMI in these two subtypes. The numbers of indels, numbers of rearrangements or rearrangement signatures showed no evidence of association with BMI. For 31 known oncogenic drivers present in at least 2% of the tumors, we observed that *CCNE1*, *FGFR1*, *IGFR1* amplifications were significantly associated with increased BMI, while *PTEN* and *CDKN2A* alterations were associated with lower BMI in triple-negative tumors. In ER+/HER2- BC, *CDH1* and *TBX3* alterations were significantly associated with increased BMI. In HER2+ BC, *MYC* amplifications were associated with lower BMI. At the transcriptomic level, BMI was positively correlated with leptin expression in postmenopausal ER+/HER2-, and with *PTEN* expression in triple-negative BC, respectively. Proliferation-based expression signatures were inversely correlated with BMI in triple-negative BC. Finally, nearly no tumor from obese patients presented a strong lymphocytic infiltration, especially in triple-negative BC.

Conclusion: This study is, to the best of our knowledge, the first report of a comprehensive genomic, transcriptomic and immune characterization of BC according to patient BMI. Although the present findings need to be validated in an external dataset, they suggest that BMI impacts BC biology and could influence targeted and immune treatment strategies especially in triple-negative BC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-03

Title: The genomic landscape of breast metaplastic carcinoma

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Body: Introduction: Metaplastic breast carcinoma (MBC) is a rare histologic type of triple-negative breast cancer (TNBC), characterized by the presence of cells displaying squamous and/or mesenchymal differentiation. The transcriptomic profiles of MBCs have been reported to vary according to the type of metaplastic elements. The somatic genetic alterations that underpin this breast cancer subtype remain to be fully characterized. Here we sought to define the genomic landscape of MBCs, whether different subtypes of MBC would be driven by distinct constellations of genetic alterations, and to investigate functionally the impact of mutations affecting WNT pathway genes using non-malignant breast epithelial cells.

Methods: Thirty-five MBCs were retrieved from the pathology department of the authors' institutions and classified into the MBC histologic subtypes. All but one of the MBCs were of triple-negative phenotype. DNA was extracted from microdissected tumor-normal pairs and subjected to whole-exome sequencing. Somatic genetic alterations were identified using state-of-the-art bioinformatics algorithms. The genomic profiles of MBCs were compared to those of 69 common type TNBCs from The Cancer Genome Atlas. Overall mutation rates were compared using the Mann Whitney U test, and the frequency of mutations in each gene was compared using Fisher's exact test. RNA was extracted from a subset of MBCs and subjected to WNT signaling pathway activation analysis with the RT² Profiler PCR Array. Triple-negative non-malignant breast epithelial cells (MCF10A and MCF12A) and cancer cell lines were utilized for 2D and 3D functional studies.

Results: Whole-exome analysis revealed that MBCs displayed a median of 103 (15-344) somatic mutations, which did not differ from the median number of somatic mutations in common type TNBCs (76, range 14-233). The most frequent recurrently mutated cancer genes included *TP53* (69%) and *PIK3CA* (29%). MBCs more frequently harbored mutations in PI3K pathway genes than common type TNBCs (57% vs 22%, $P < 0.05$), including mutations affecting *PIK3CA* (29% vs 7%), *PIK3R1* (11% vs 0) and *PTEN* (11% vs 1%). MBCs also more frequently harbored mutations affecting WNT signaling pathway genes (46% vs 26%, $P < 0.05$), including *AXIN1* (6% vs 1%), *WNT5A* (6% vs 0) and *APC* (3% vs 0). MBC subtype analysis revealed that *PIK3CA* mutations were only detected in non-chondroid MBCs (53% vs 0), *CHERP* mutations were only found in chondroid MBCs (25% vs 0), whereas *USP5* mutations only found in squamous MBCs (33% vs 0). MBCs with somatic mutations in WNT pathway genes had significantly higher WNT pathway activation than MBCs lacking mutations in these genes ($P = 0.0244$). Consistent with the mesenchymal phenotype frequently exhibited by MBCs, *in vitro* experiments provided functional evidence that aberrant WNT pathway activation induces an epithelial-to-mesenchymal transition (EMT) phenotype, with downregulation of epithelial markers and upregulation of EMT transcriptional inducers.

Conclusions: MBCs are significantly enriched for mutations affecting PI3K and WNT pathways, highlighting the importance of the dysregulation of the WNT pathway in MBC carcinogenesis. Moreover, our findings suggest that specific mutations are significantly associated with distinct histologic subtypes of MBCs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-04

Title: Intra-tumor genetic heterogeneity and histologic heterogeneity within metaplastic breast cancers: Genotypic-phenotypic correlations

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Body: Introduction: Metaplastic breast carcinoma (MBC) is characterized by the presence of neoplastic cells displaying squamous and/or mesenchymal differentiation. Morphologic intra-tumor heterogeneity is frequent in MBCs and reported to be reflected at the transcriptomic level: whilst squamous and chondroid MBCs are preferentially of basal-like subtype, spindle cell MBCs are of claudin-low subtype. Likewise, histologically distinct components within MBCs have been shown to display distinct focal copy number alterations. Here we sought to investigate whether histologically distinct components within MBCs would be underpinned by different mutational profiles and mutational signatures.

Methods: Ten MBCs with two histologically distinct components (spindle, chondroid, osseous, squamous and/ or ductal) were retrieved from the Department of Pathology of the authors' institution. The distinct components of each case and, in two cases, two regions of the same component were separately microdissected. DNA extracted from tumor samples (n=22) and matched normal tissues was subjected to whole-exome sequencing. Somatic genetic alterations were identified using state-of-the-art bioinformatics algorithms. Somatic mutations were classified as clonal (i.e., present virtually in all tumor cells) or subclonal using ABSOLUTE and FACETS. Mutational signatures were defined using non-negative matrix factorization.

Results: Medians of 146 (56-290) somatic mutations and 108 non-synonymous somatic mutations (39-222) per tumor component were identified. The histologically distinct components of each case harbored identical clonal *TP53* mutations. Additional recurrent mutations in cancer genes included those affecting PI3K pathway genes (*PIK3CA*, 2 cases; *PIK3R1*, 2 cases). Shared mutations between components of each case ranged from 34% to 99% of all mutations, with a median of 84%, of which 24% (12%-53%) were truncal (i.e., shared by and clonal in both components). Private mutations (i.e., found in only one component) ranged from 1% to 66%, with a median of 16%, of which 72% (0-100%) were non-synonymous and 1% (0-52%) were clonal. In two cases, the comparison of two histologically similar regions revealed less heterogeneity, with 94% (87%-100%) of shared mutations, whereas in these samples the median of private mutations was 6% (0-13%), of which 70% (0-100%) were non-synonymous and none were clonal. Private non-synonymous mutations affecting cancer genes included those in *PIK3R1*, *MED12* and *NOTCH1*. The mutational signatures (e.g. aging or BRCA) were concordant between distinct components of each case; however, differences in the mutational signatures were observed between truncal somatic mutations and mutations restricted to individual components.

Conclusions: MBCs display substantial genetic intra-tumor heterogeneity, which is more overt between histologically distinct components than between regions of similar histology. Our data suggest a genotypic-phenotypic correlation and corroborate the notion that distinct components within MBCs, although clonally related, may be driven by distinct somatic genetic alterations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-05

Title: Comparison of genotyping results from tissue and circulating DNA (ctDNA) in patients with metastatic breast cancer

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Body: Introduction: Robust clinical genotyping assays are crucial to accelerate development of targeted therapies toward tumor-specific oncogene pathways. Indeed, there has been an explosion in the development of genotyping assays and stratified enrollment in clinical trials based on molecular characteristics of the tumor. However, availability of tissue can be a significant barrier for genotyping, particularly patients with estrogen receptor positive (ER+) breast cancer, who frequently have bone only metastasis. Detection of mutations in circulating tumor DNA (ctDNA) represents an attractive alternate strategy. However, there is a paucity of data comparing genotyping results from tissue and ctDNA, and potential clinical utility for patients with metastatic breast cancer.

Methods: Analysis of ctDNA was based on Guardant 360 panel (2015), a next-generation sequencing (NGS) based assay that covers point mutations in 70 genes (complete or critical exon coverage) with select amplifications, fusions, and indels, using NGS Illumina HiSeq platform, validated to detect alterations in samples with at least 2% ctDNA. Molecular profiling of tissue was based on the institutional lab-developed test "Snapshot-NGS assay", utilizing a multiplex polymerase chain reaction (PCR) technology called Anchored Multiplex PCR (AMP) for single nucleotide variant (SNV) and insertion/deletion (indel) detection in genomic DNA targeting hotspots and exons in 39 genes using NGS Illumina MiSeq platform, validated to detect SNV and indel variants at 5% allelic frequency or higher in target regions.

Results: In the analytical dataset, a total of 42 patients with metastatic breast cancer (median age 62) at our institution had ctDNA and tissue Snapshot-NGS results available. Out of these, 95% had at least one genetic alteration detected in ctDNA, before start of a new therapy. The common genetic alterations in ER+ disease (n = 33) included *PIK3CA* (39.4%), *ESR1* (27.3%), and *AKT1* (15.2%); HER2+ disease (n = 2) included *PIK3CA* and *CCND1*; TNBC (n = 7) included *TP53* (71.4%). Gene amplifications in *FGFR*, *PIK3CA*, *CCND1*, *MYC*, and *KRAS* were also noted. In general, the average number of molecular alterations was higher in ctDNA than the tissue specimen (mean = 5.1 vs 1.1; p < .001). Of 34 patients with actionable alterations detected in ctDNA, 76.5% of these patients did not have actionable alterations detected in tissue. Of 29 patients with actionable alterations detected in tissue (primary or metastatic), 13.8% of these patients did not have any actionable alterations detected in ctDNA.

Conclusion: A significantly higher number of genomic alterations were detected in ctDNA compared to tissue, including actionable alterations linked to potential targeted therapies. Given these findings and the difficulties with tissue-based genotyping assays, blood based biomarker assays might provide an alternative and effective way to obtain a comprehensive molecular profile, and potentially guide management decisions for patients with metastatic breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-06

Title: Estrogen receptor 1 (*ESR1*) mutations in circulating tumor DNA (ctDNA): A guide to the management of advanced breast cancer (ABC)

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Body: Background: Estrogen receptor (ER)- α is expressed in about 70% of breast cancers and drugs that target the receptor function, selective estrogen receptor modulators (SERM) and aromatase inhibitors (AIs) represent the standard of care for patients (pts) with ER+ breast cancer. Nevertheless, prolonged exposure to endocrine therapy may result in acquired resistance and subsequent progression of disease. Recent evidence showed that activating mutations (muts) in the ligand-binding domain of ER- α occur in approximately 20% of pts exposed to endocrine therapies and those genomic abnormalities may represent the driver of endocrine resistance. In this context, ctDNA provides a non-invasive source for *real-time* next generation sequencing (NGS) studies, in order to understand the biology of ABC and guide and monitor treatment.

Methods: We conducted a retrospective review of 91 pts with ABC, including 57 pts with ER+ tumor, who had longitudinal assessment of their disease by ctDNA analysis. At the time of baseline sampling, 50/57 pts had stage IV cancer. The total number of blood samples collected was 184. 38 (67%) pts had serial samples. The average number of samples for each pt was 3 (range 1-7). The plasma-based assay was performed utilizing Guardant360 (Guardant Health, CA), a digital NGS technology to sequence a panel of > 50 cancer genes.

Results: Among the ER+ subgroup (57 pts), we identified 11 pts (19%) harboring *ESR1* muts in ctDNA. All 11 pts had metastatic disease: 2 (18%) had bone metastases, 2 (18%) had visceral metastases, 7 (64%) had both sites of disease. The median age was 55 years (range 33-73). 5 pts had inflammatory breast cancer. The most common *ESR1* muts were: Y537S (6/11, 55%), D538G (4/11, 36%) and Y537N (3/11, 27%). 7 pts carried polyclonal muts. At the time of testing, 10 pts had already failed at least 1 line of endocrine therapy (average 2, range 1-5), including 6 pts that had received a fulvestrant-containing regimen, 8 pts \geq 1 line of AIs. After the mut detection, 5 pts were on endocrine therapy and 4 pts were started on/continued chemotherapy. *ESR1* muts disappeared in 2 pts (fulvestrant-palbociclib and chemotherapy respectively) who achieved stable disease as best response. Three pts continued to harbour muts and then progressed (one died). 2 pts had tissue NGS and *ESR1* mut was not identified. Progression free survival and overall survival were 8 months (ms) and 21.5 ms in *ESR1*+ subpopulation *versus* 6.2 ms and 22.2 ms in the *ESR1*- pts ($p = 0.78$ and $p = 0.97$, respectively). At the time of analysis 5 pts were dead, 6 were currently alive.

	<i>ESR1</i> + (n. pts)	<i>ESR1</i> - (n. pts)
Pts (total n.)	11	46
Previous chemotherapies	11 (100%)	31 (67%)
Previous fulvestrant-containing regimens	6 (54%)	20 (43%)
Previous AIs \pm targeted therapy	8 (73%)	27 (59%)

Conclusions: We observed that *ESR1* muts, a known driver of endocrine resistance, occurs at a high frequency in heavily pre-treated estrogen receptor positive ABC. Blood-based diagnostics can be used to identify *ESR1* muts sometimes not detected by tissue-based sequencing of the metastatic lesions indicating tumor heterogeneity and allowing dynamic monitoring of ABC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-07

Title: Comprehensive genomic profiling of clinically malignant phyllodes tumors of the breast reveals frequent mutation of *NF1* and other genes associated with PI3K and RAS pathway activation

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Body: Background: Malignant or metastatic breast phyllodes tumors (MPT) are exceptionally rare, and the underlying genomic drivers are still being elucidated. Recent studies report frequent mutations in the RAS and PI3K pathways but have not commonly reported mutations in *NF1*. Comprehensive genomic profiling (CGP) can measure mutation load (TMB) and identifies all four classes of oncogenic alterations, including rearrangements and copy number loss that commonly affect tumor suppressors such as *NF1*, and can direct personalized treatment strategies.

Methods: CGP using hybridization capture of 3,769 exons from up to 315 cancer-related genes and select introns of 28 genes commonly rearranged in cancer was applied to ≥ 50 ng of DNA extracted from 21 consecutive MPT and sequenced to high, uniform median coverage ($>400\times$). TMB was determined as mutations/Mb on 1.1 Mb of sequenced DNA.

Results: The 21 MPT featured a median age of 51 yrs (range 14-70 yrs). CGP was performed on the primary MPT in 15 cases and on metastasis biopsies in 6 cases. TMB for all MPT was low (<10 mut/Mb), and all evaluable tumors (17/21) were microsatellite stable (MSS). The most commonly mutated genes were *TP53* (57.1%), *TERT* (56.3%), *NF1* (52.4%), *MED12* (38.1%), *CDKN2A/B* (33.3%), and *MLL2* (33.3%). 19/21 (90.5%) MPT harbored clinically relevant genomic alterations (CRGA) associated with therapies available on the market or under investigation in late stage clinical trials. Additional alterations in the PI3K/AKT/MTOR, RAS/RAF/MEK, and FGFR pathways were identified (see table); the PI3K/ATK/MTOR pathway was mutated in 10/21 (47.6%) of samples. Although *CDKN2A/B* loss was found in 6/11 tumors with *NF1* mutation and only 1/10 *NF1* wild-type samples, the co-occurrence was not significant ($p<0.07$). No significant correlation exists between the occurrence of *NF1* mutations and mutation of *MED12*, *TERT*, the PI3K pathway, or other genes in the RAS/RAF pathway (*NRAS*, *BRAF*, *EGFR*). Targetable *KIAA1549-BRAF* or *FGFR3-TACC3* fusions were identified in 2/21 (9.5%) tumors. Responses to targeted treatments will be presented.

Conclusions: More than 90% of MPT feature CRGA, including alteration of *NF1*, which was by far the most common targetable GA in this study. 52.4% of MPT had alterations predicted to result in loss of *NF1* activity. *NF1* mutation does not significantly co-occur with mutations in any other gene or pathway commonly altered in MPT. Other tumors with underlying *NF1* mutations have responded to the MEK inhibitor selumetinib, suggesting MEK inhibitors may be relevant for the treatment of MPT. Other targetable alterations, including known gene fusions, are common in MPT. Thus, MPT may benefit from combination targeted therapy, warranting further investigation in the clinical trial setting.

Pathway	Total Cases (n=21)	Total Mutation Number		
		Short Variants	Copy Number	Rearrangements
RAS/RAF/MEK				
<i>NF1</i>	11 (52.4%)	7	2	2
<i>BRAF</i>	3 (14.3%)	3	0	1
<i>NRAS</i>	2 (9.5%)	3	0	0
PI3K/AKT/MTOR				
<i>PIK3CA</i>	4 (19%)	3	1	0
<i>PTEN</i>	4 (19%)	1	3	0
<i>STK11</i>	2 (9.5%)	1	1	0
<i>AKT1</i>	1 (4.8%)	1	0	0

<i>FBXW7</i>	1 (4.8%)	0	0	1
<i>TSC2</i>	1 (4.8%)	0	1	0
<i>PIK3R1</i>	1 (4.8%)	1	0	0
FGFR				
<i>FGFR1</i>	1 (4.8%)	1	0	0
<i>FGFR3</i>	1 (4.8%)	0	0	1
Other				
<i>EGFR</i>	2 (9.5%)	0	2	0
<i>BRCA2</i>	1 (4.8%)	1	0	0
<i>PDGFRA</i>	1 (4.8%)	0	1	0
<i>KIT</i>	1 (4.8%)	0	1	0

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-08

Title: Comprehensive genomic profiling of 8,654 breast carcinoma reveals therapeutically targetable molecular subtypes beyond those defined by hormone-receptor expression

Ross JS S, Gay LM M, Elvin JA A, Suh J, Vergilio J-A, Ramkissoon S, Schrock A, Ali S, Miller VA A and Stephens PJ J. Foundation Medicine, Inc., Cambridge, MA and Albany Medical College, Albany, NY.

Body: Background: Breast carcinomas (BC) are commonly classified into 4 subtypes based on hormone receptor expression: basal, luminal A, luminal B, and HER2 overexpressed. Comprehensive genomic profiling (CGP) reveals targetable genomic alterations (GA) across all four mutation classes, as well as measuring tumor mutational burden (TMB), and can redefine BC classification into therapeutically relevant subtypes. Testing with immunohistochemistry or hotspot testing can miss a substantial number of targetable alterations and cannot measure TMB.

Methods: DNA was extracted from 40 µm of FFPE sections for 8654 consecutive BCs. CGP was performed on hybridization-captured, adaptor ligation-based libraries (mean coverage >500X) for up to 315 cancer-related genes and select introns from up to 28 genes frequently rearranged in cancer. Sequences were analyzed for substitutions, small insertions/deletions, copy number changes, and rearrangements. TMB was determined by counting non-driver, non-germline alterations across 1.1 Mbp of sequenced DNA. Clinically relevant GA (CRGA) are GA linked to therapies on the market or under evaluation in clinical trials. Immunotherapy (IO) sensitivity is defined as TMB >20 mut/Mbp or mutation of specific DNA repair pathways.

Results: The table below outlines 7 distinct functional or signal transduction pathways commonly altered in BC. Several are targetable with therapies that are FDA approved for an oncology indication. Mutations can also be found in other targetable kinases such as RET, ROS1, and RAF. 6959 (80.4%) tumors harbor a GA in at least one pathway, and 2697 (31.2%) BC harbor alterations in just one pathway (unique cases). Only 9.8% of BC would be HER2-positive by IHC. Almost 4% (352/8654) of cases harbor rearrangements or gene fusions that may not be detectable with other assays. Mutations in *ESR1* characterize an eighth category of tumors with acquired resistance to endocrine therapy; 796/8654 (9%) samples harbor *ESR1* alterations.

Conclusions: CGP can identify CRGA and TMB that can stratify tumors by predicted sensitivity to a variety of therapies, including HER2- or mTOR-targeted therapies, immunotherapies, and other kinase inhibitors. 80% of BC harbor targetable GA, and 30% of samples harbor mutations in only one pathway. CGP can provide crucial information for identifying which of several treatment modalities is most appropriate for these 30% of patients. High levels of TMB and most GA would not be identified by IHC or hotspot testing, but can be detected by next-generation sequencing. CGP is a powerful tool for guiding treatment across therapeutically distinct, but targetable, pathways.

	PI3K/AKT/mTOR pathway	FGFR pathway	CDK pathway	ERBB pathway	HR deficient	IO sensitive	Other kinases
Total Cases	4375	2650	2685	1294	1266	419	424
% Total Cases	51%	31%	31%	15%	15%	5%	5%
Unique Cases	1442	226	231	274	309	48	58
% Unique Cases	17%	3%	3%	3%	4%	1%	1%
Therapies	Everolimus, Temsirolimus	Pazopanib, Ponatinib	Palbociclib	Trastuzumab, Pertuzumab, Afatinib, Lapatinib, Neratinib	Olaparib	Pembrolizumab, Nivolumab, Atezolizumab, Ipilimumab	Sorafenib, Regorafenib, Dabrafenib, Vemurafenib, Crizotinib,

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-09

Title: Association of co-amplicons with immune infiltration in subtypes of HER2-Positive breast cancer

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Body: Background: HER2+ breast cancers are heterogeneous at both clinical and molecular levels. We and others have determined that the HER2-Enriched subtype exhibits the highest rate of pathologic complete response (pCR) to neoadjuvant chemotherapy and trastuzumab (T), while the HER2-Basal subtype is resistant to anti-HER2 therapy (Carey et al, JCO 2015; Varadan et al, CCR 2016). Additionally, we reported that signatures of immune cell infiltration and immune cell subsets evaluated after one dose of T can predict pCR to preoperative T and chemotherapy (Varadan et al, CCR 2016). Given recent evidence for improved immune response with increasing mutational load, we chose to characterize the association of somatic mutations and copy-number alterations with subtypes of HER2+ breast cancer and immune modulation after one dose of T.

Methods: Fresh tumor core biopsies were taken at baseline and 2 weeks after one dose of either T or nab-paclitaxel (N) from 60 patients with stage II-III HER2+ cancers enrolled on a multicenter trial (BrUOG 211B). All patients then received 18 weeks of T+N+carboplatin. PAM50 subtyping was performed using gene expression data from patient tumor biopsies and tumors were classified into HER2-Enriched, HER2-Luminal and HER2-Basal subtypes. Whole-exome sequencing (WES) was performed on a total of 86 samples (49 baseline, 37 brief-exposure), sequenced at an average depth of 90X. Somatic mutations were detected by applying multiple mutation-detection algorithms on the WES data, followed by stringent quality control using public and in-house variant databases, and mutation data curated from 11,000 tumors sequenced by the TCGA. Somatic copy-number alterations were estimated using a published algorithm, ENVE (Varadan et al, Genome Med 2015) that robustly detects somatic copy-number alterations in WES tumor profiles. We employed previously defined gene-expression signatures (Varadan et al, CCR 2016) of total immune infiltration and immune cell subsets, to assess for association with genomic aberrations.

Results: HER2-Basal tumors exhibited lower average copy number for HER2 and were less likely to have high-level amplifications of co-amplicons (e.g. 11q13, 20q13) with the exception of the MYC amplicon (8q24). They also exhibited a non-significant ($P=0.33$) trend towards higher mutational burden (Avg=85) compared to HER2-Luminals (Avg=79). A majority of somatic mutations (62%, 2282/3666) persisted after a single-dose of either T or N, while 17% (624/3666) were not detectable after brief-exposure. There was no association between immune infiltration and mutational burden in any HER2 subtype. Tumors harboring *FGFR1* (8p11) amplifications exhibited higher gene-signature levels for macrophages ($P=0.0073$) and T-cells ($P=0.0493$) but not B-cells ($P=0.213$).

Conclusions: The HER2-Basal subtype is less likely to respond to trastuzumab-based neoadjuvant therapy and exhibits lower numbers of common amplicons. The disappearance of mutations after brief-exposure to therapy may be due to either tumor heterogeneity/sampling or clonal selection. The association of 8p11 amplifications with increased T-cell infiltration suggests that this amplicon may play an immunogenic role in HER2+ breast cancer. These results warrant further investigation in larger cohorts.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-10

Title: MLL3 mutation in ER+ breast cancers leads to dramatic shift in ER binding locations and consequentially alters transcriptional programs

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Body: Hypothesis:

Endocrine therapy (ET) is an effective treatment for estrogen-receptor positive (ER+) breast cancer; however, more than 80% of women will develop endocrine resistance. ER ChIP-seq studies in clinical samples show tumors likely to relapse have a unique set of ER binding sites that correlate with gene signatures predicting clinical outcome. Understanding how several factors, including transcriptional co-activator-complexes, influence ER binding location is crucial to improving treatment. MLL3, which is recurrently mutated in ~7% of ER+ cases, is a member of one such complex that interacts with ER. It is a histone methyltransferase that marks active enhancers. We hypothesized that mutation of MLL3 may change the genomic landscape of enhancers and thus change binding patterns of ER, altering response to ET.

Methods:

To elucidate transcriptional consequences of MLL3 mutation in ER+ breast cancers, we leveraged TCGA ER+ breast cancer RNA-seq data. We used a gene-by-gene multivariate linear model to identify differentially expressed genes (DEGs) between MLL3-mutant and wild-type (WT) samples. This data was compared to DEG found with in-house RNA-seq data for MLL3-mutant MCF7 cells, MLL3-WT ZR751 cells, and MLL3-knockdown (KD) ZR751 cells to identify a testable MLL3-mutant signature. ChIP-seq data for ER and H3K4me1, the histone mark made by MLL3, was then produced using these cell lines in order to pinpoint genes with changes in both H3K4me1 and ER-binding upon MLL3 mutation/KD. This gene list was compared to DEG found through RNA-Seq. To illuminate inherent differences between MLL3-WT and mutant/KD cells in response to ET, Cell-Titer Blue, Cell-Titer Glo, and Caspase-Glo assays were performed using DMSO, 4-OHT, and Fulvestrant.

Results:

TCGA RNA-seq data revealed MLL3-mutant ER+ breast cancers have differentially expressed transcriptional regulators, including ER itself. iRegulon analysis showed enrichment for DEG-regulated by ER-tethering factor SP1, a phenomenon suggested to be associated with more aggressive behavior. ChIP-seq data revealed substantial shifts in both ER-binding locations and H3K4me1 marks upon MLL3 mutation/KD. Interestingly, annotated peaks for MLL3-KD ZR751 showed overlap with those of MLL3-mutant MCF7, and GREAT analysis of new ER binding sites in MLL3-KD ZR751 showed an up-regulation of signatures associated with endocrine resistance. Indeed, MLL3-KD ZR751 cells were more resistant to 4-OHT/Fulvestrant than MLL3-WT ZR751.

Conclusions:

Loss of MLL3 function leads to a massive shift in H3K4me1, indicating a shift in the genomic landscape of enhancers. This shift is associated with a shift in ER binding as well as alterations in gene expression. Pathway analysis of these genes suggest that loss of MLL3 function may increase endocrine resistance, and indeed, cell lines with loss of MLL3 function are more resistant to ER inhibition than MLL3-WT cell lines. This work demonstrates that loss of MLL3 function leads to shifts in the enhancer landscape, alterations in ER binding and regulation of gene expression, and contributes to more aggressive tumor behavior.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-11

Title: Comprehensive comparison of breast cancer molecular portraits by African and European ancestry in the cancer genome atlas

Huo D, Hu H, Rhie SK K, Gamazon ER R, Cherniack AD D, Liu J, Yoshimatsu TF F, Pitt JJ J, Hoadley KA A, Troester M, Ru Y, Lichtenberg T, Sturtz LA A, Shelley CS S, Mills GB B, Laird PW W, Shriver CD D, Perou CM M and Olopade OI I. University of Chicago; Chan Soon-Shiong Institute of Molecular Medicine at Windber; University of Southern California; Vanderbilt University; The Eli and Edythe L. Broad Institute of MIT and Harvard; University of North Carolina at Chapel Hill; Nationwide Children's Hospital, Columbus; University of Wisconsin; University of Texas MD Anderson Cancer Center; Van Andel Research Institute and Walter Reed National Military Medical Center.

Body: Background: African American breast cancer patients have worse survival rates than European American patients. Although racial differences in the distribution of breast cancer intrinsic subtype are known, it is unclear if there are other inherent genomic differences contributing to this racial outcome disparity.

Methods: We defined patient race based on genomic ancestry and compared multiple molecular features of breast cancer between 154 black and 776 white patients in The Cancer Genome Atlas (TCGA). We examined the contribution of these molecular features to survival outcomes using Cox proportional hazards models. We also estimated the heritability of breast cancer subtypes using a mixed effect model.

Results: Compared to whites, black patients had higher odds of basal-like (odds ratio=3.80, $p<0.001$) and HER2-enriched (odds ratio=2.22, $p=0.027$) breast cancers in reference to luminal A subtype. Beyond differences in relative frequency of intrinsic subtypes, black and white patients had distinct gene expression, protein expression, and somatic mutation landscapes. However, the majority of these molecular differences were eliminated after adjusting for subtype; in the subtype-adjusted models, we found 142 genes, 16 methylation probes, 4 copy number segments, 1 protein, and no somatic mutation were differentially expressed or present between black and white patients. Using the top 40 differentially expressed genes, we built a race-enriched gene signature, which had excellent capacity of distinguishing breast tumors from black versus white patients (c-index=0.852 in the validation dataset). We also estimated the heritability of breast cancer subtype (basal vs. non-basal) to be 0.436 ($p=1.5\times 10^{-14}$) and showed that two genetic variants (rs1078806 in *FGFR2*, rs34084277 in *BABAM1*) were associated with intrinsic subtype and can partially explain racial differences in subtype frequencies.

Conclusion: On the molecular level, once intrinsic subtype frequency differences are accounted for, there are few genomic or proteomic differences observed between blacks and whites. More than 40% of breast cancer subtype frequency differences may be due to genetic ancestry. These results suggest that future studies are warranted to investigate genetic and non-genetic factors that contribute to the development and progression of breast cancer subtypes in order to reduce racial disparity.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-12

Title: Clonal heterogeneity in breast cancer and its impact on clinical biomarkers

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Body: Introduction: Clonal heterogeneity in cancer is associated with resistance to therapies and evolution of metastatic disease. Clinical management of breast cancer relies on the status of estrogen (ER), progesterone (PR), and Her2 receptors in diagnostic biopsies. Results for these biomarkers rely on IHC and FISH assays that incorporate staining intensity and percentage of positive cell numbers for each sample of interest. Heterogeneous results for one or more of these biomarkers suggest the presence of multiple tumor populations within a biopsy. However the impact of clonal heterogeneity on clinical biomarkers has not been rigorously evaluated.

Methods: We interrogated the clonal composition of 3 treatment-naïve surgically excised invasive ductal carcinomas. Patient #1 cancer, (pT2, pN0) was grade 3, ER+, PR+, and Her2-- with a germ line *BRCA2*^{Q49X} mutation. Patient #2 cancer (pT2, pN0) was grade 3, ER+ PR- Her2+. Patient #3 was a locally advanced cancer with both axillary and supraclavicular lymph node (LN) involvement (pT2, pN3c, and was grade 3, ER+PR-Her2+ by core but found to be ER+PR-Her2- on the resection specimen. We applied DNA content flow cytometry to multiple (8-18) mapped biopsies in each case. Each sorted tumor population was interrogated with whole exome sequencing, and whole genome array comparative genomic hybridization (aCGH). Single cell analysis of aneuploid populations present in the primary tumor and lymph nodes in patient 3 was done to further assess clonal heterogeneity.

Results: Tumor from patient #1 had a single ploidy with stable copy number profiles including an interstitial 13q deletion that converted a germ line *BRCA2*^{Q49X} mutation to homozygosity, and a clonal homozygous deletion in *Numb* in each of 4 tumor biopsies. Tumor from patient #2 had a dominant ploidy (3.2N) in each of 10 (A1-A10) primary tumor biopsies but with a second co-existing ploidy (3.6N) in one (A2) biopsy. A homozygous somatic *BRCA2*^{r3129x} mutation was identified in all 10 biopsies and in both ploidies; however the genomes had heterogeneous aCGH profiles. In contrast, 12 primary biopsies from Patient #3 contained 6 distinct ploidies with highly aberrant but homogenous genomes, characterized by *SARC* amplification, homozygous deletion of *ROBO1* and *ROBO2*, and clonal mutations in *TP53*, *NF1*, and *PIK3CA*. One of the ploidies, (5.8N) was present in an adjacent node and another (5.0N) in both the adjacent and distant nodes. Single cell analyses of the 5.0N and 5.8N populations revealed that allele frequencies of driver mutations were stable in the tumor and become homozygous in the LNs. There were no *Her2* amplicons or mutations in biopsies analyzed in Patients #2 and #3 further contradicting the heterogeneous staining for ER, PR and Her2.

Conclusion: Tumor heterogeneity includes variations in ploidies, copy number aberrations, and allele frequencies. However even highly aberrant aneuploid genomes can be stable within multiple primary and lymph node sites. Rigorous interrogation of flow sorted tumor populations, in contrast to inferring heterogeneity in bulk samples, identifies driver aberrations in single biopsies, challenges heterogeneous results with clinical biomarkers, and distinguishes allelic heterozygosity from tumor heterogeneity in highly aberrant aneuploid tumor genomes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-13

Title: The metastatic breast cancer project: Translational genomics through direct patient engagement

Painter CA A, Anastasio E, Krevalin M, Kim D, Larken K, Lennon N, Frank E, Winer EP P, Lander ES S and Golub T. Broad institute of MIT and Harvard, Cambridge, MA and Dana-Farber Cancer, Boston, MA.

Body: Background: The Metastatic Breast Cancer Project is a nationwide research initiative that directly engages patients through social media and advocacy groups and seeks to empower them to share their samples and clinical information to accelerate research. Because the vast majority of patients are treated in the community setting, we sought to determine the feasibility of remotely obtaining tumor and saliva samples as well as medical records from a large cohort of metastatic breast cancer (MBC) patients who receive their care in diverse settings around the country.

Methods: In collaboration with patients and advocacy groups, we developed a website to allow MBC patients across the U.S. to participate. Enrolled patients are sent a saliva kit and asked to mail back a saliva sample, which is used to extract germline DNA. We contact participants' medical providers and obtain medical records and part of their tumor biopsy. Whole exome and transcriptome sequencing is performed on tumor and germline samples. Clinically annotated genomic data are used to identify mechanisms of response and resistance to therapies. The database will be shared widely with researchers. Study updates and discoveries are shared with participants regularly.

Results: In the first 8 months, 2285 MBC patients from all 50 states enrolled. 2163 (95%) completed the 16-question survey about their cancer, treatments, and demographic information. 1232 completed the online consent form permitting acquisition and analysis of medical records, tumor tissue, and saliva samples. 556 saliva samples have been received. Initial medical record and tumor sample requests have been made for patients who have provided saliva samples. To date, we have obtained medical records from 102 patients (93% success rate) and tumor samples from 32 patients (77% success rate). Whole exome and transcriptome sequencing has been successfully completed on initial samples received and is ongoing for additional samples.

Conclusions: Partnering directly with patients through social media and advocacy groups enables rapid identification of thousands of patients willing to share tumors, saliva, and medical records to accelerate research. This approach allows for rapid identification of patients with rare phenotypes such as extraordinary responders, who have been challenging to identify with traditional approaches. Remote acquisition of medical records, saliva samples, and tumor tissue for patients located throughout the U.S. is feasible. Genomic analysis and medical record abstraction for these patients is underway. As data is generated, a clinically annotated database will be shared widely with the research community.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-14

Title: Copy number aberration-induced gene breakage analysis identifies recurrent *FOXP1* fusions in breast cancer

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Body: Background: Genomic instability is a critical feature of breast cancers, which manifests in genome-wide copy number aberrations (CNA), often causing “gene breakage” and the generation of fusion genes. We aimed to identify aborted transcripts with underlying CNAs and to investigate the molecular landscape of breast cancers harbouring such events.

Methods: A walking student's *t*-test algorithm was applied to Affymetrix Exon 1.0ST array data of 123 breast cancers to identify regions of aborted transcription and overlaid with DNA breakpoints derived from matched Affymetrix SNP6 ASCAT-segmented copy number. Aborted transcripts were investigated as potential fusion gene partners through RNA-seq analysis of 151 breast cancer samples (TCGA) and 51 breast cancer cell lines (BCCL) using ChimeraScan. Clinical correlates were established for clinicopathological features, genomic instability measures, and gene expression-based molecular classifiers including PAM50, TNBCtype, IntClust subtypes and immune signatures.

Results: One hundred and six genes with recurrent CNA-induced aborted transcription were identified. Aborted transcription showed hormone receptor subtype-specificity for 7 genes ($n_{\text{TNBC}}=1$, $n_{\text{Non-TNBC}}=6$) and was less prevalent in samples of IntClust 2 and IntClust 4 subtypes (p : 0.0043, 0.0011). Aborted transcripts were more frequently observed in samples with greater copy-neutral loss of heterozygosity ($p=0.012$), while aborted transcription of 54/106 genes significantly affected enrichment of 27 tumor-infiltrating lymphocyte subpopulations. 14 aborted transcripts were found as a fusion gene with one partner in RNA-seq of TCGA and BCCL, while 19 were involved in multiple fusion events (range=1-6, median=2). Nine of 106 genes displayed gene breakage and fusion events exclusively in samples with an enriched tandem duplication phenotype. Notably, *FOXP1*, localised to a tumour suppressor locus at 3p14.1, reported the highest number of fusion configurations ($n=6$) with concurrent aborted transcription across all RNA-seq datasets ($n_{\text{PRADA}}=9$, $n_{\text{TCGA}}=38$, $n_{\text{BCCL}}=6$).

Conclusion: CNA-induced gene breakage affects the molecular landscape of breast cancers and is linked with many genomic configurations of interest including copy-neutral loss of heterozygosity and tandem duplications. In particular, the role of recurrent gene fusions of the tumour suppressor, *FOXP1*, in tumourigenesis warrants further investigation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-15

Title: Multi-omics and immuno-oncology profiling reveal distinct molecular signatures of young Asian breast cancers

Kan Z, Ding Y, Cho S, Lee S-H, Powell E, Jung HH, Chung W, Deng S, Choi Y-I, Kim J, Park W-Y, Vizcarra P, Fernandez-Banet J, Nichols T, Ram S, Lee SK, Kim SW, Lee JE, Ching KA A, Kim J-Y, Ahn JS, Im Y-H, Nam SJ and Park YH. Pfizer Inc., San Diego, CA and Samsung Medical Center, Seoul, Korea.

Body: Breast cancers (BC) in younger, premenopausal patients (YBC) tend to be more aggressive with worse prognosis, higher chance of relapse and poorer response to endocrine therapies compared to breast cancers in older patients. The proportion of YBC (age \leq 40) among BC in East Asia is estimated to be 16-32%, significantly higher than the 7% reported in Western countries. To characterize the molecular bases of Asian YBC, we have performed whole-exome sequencing (WES) and whole-transcriptome sequencing (WTS) on tumor and matched normal samples from 134 Korean BC patients consisting of 74 YBC cases (age \leq 40) and 60 OBC cases (age $>$ 40). We then performed comparison analyses and integrative analyses with the TCGA BC cohort consisting of 1,116 tumors from primarily Caucasian patients, also grouped by age into YBC (age \leq 40), IBC (40 $<$ age \leq 60) and OBC (age $>$ 60).

Somatic mutation prevalence analysis identified 7 significantly mutated genes and the same top three genes – *TP53*, *GATA3* and *PIK3CA* – were reported by the TCGA BC study. To identify differentially expressed (DE) genes and pathways in YBCs vs. OBCs, we performed logistic regression analyses while controlling for the confounding effects of tumor purity and stage. We were surprised to see a significant overlap in DE pathways between a comparison of adjacent normal tissues in younger vs. older TCGA cohorts and a comparison of YBC vs. OBC tumors, indicating that normal tissue compartment could contribute to observed differences between bulk tumors. To separately examine molecular signatures from tumor, stroma and normal compartments, we used non-negative matrix factorization (NMF) analyses to virtually dissect bulk tumor expression data and identified 14 factors including 3 factors associated with normal tissues, 1 factor associated with stroma and 1 factor associated with tumor infiltrating lymphocytes (TIL). Integrative analyses of tumor associated factors and DE pathways revealed that estrogen response, endocrine therapy resistance, and oxidative phosphorylation pathways are up-regulated in YBCs compared to OBCs while cell cycle and proliferation pathways are up-regulated in Asian OBCs. Interestingly, many immune and inflammation pathways correlated with the TIL factor were significantly upregulated in OBCs vs. YBCs. Using gene expression signatures representing distinct immune cell types, we classified our cohort into four subtypes of varying TIL activities and observed significant enrichment of the TIL-high subtype in OBCs compared to YBCs. These observations were confirmed by IHC analyses of four TIL markers (*CD45*, *CD4*, *CD8* and *CD163*) in 120 tumors.

To our knowledge, this is the first large-scale multi-omics study of Asian breast cancer and would significantly contribute to the compendium of molecular data available for studying young breast cancers. The major landmarks in the molecular landscape looked similar across BCs of different ethnicities and ages, however, we have identified a number of distinguishing molecular characteristics associated with Asian YBC. The sources for some signatures were further traced to non-tumor intrinsic compartments, indicating that tumor microenvironment may play potentially important roles in driving the carcinogenesis of young breast cancers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-16

Title: An integrated molecular analysis of invasive lobular carcinoma

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Body: Background: Invasive lobular carcinoma (ILC) is the most commonly diagnosed special histological type of breast cancer, accounting for up to 15% of all cases. Accumulating data suggests the biology and clinical features of ILC differ to those of the more commonly diagnosed Invasive Carcinoma of No Special Type (IC-NST), including evidence of there being an overall worse long term outcome associated with ILC. Several large-scale molecular profiling studies have recently been published regarding ILC, highlighting molecular heterogeneity and important drivers of tumour behaviour. We hypothesize that an integrative analysis of gene expression and DNA copy number data will identify novel drivers, and prognostic and predictive biomarkers of lobular phenotype.

Methods: Gene expression and copy number data from ILC tumours profiled in-house (n=25), METABRIC (n=125) and TCGA (n=145) were assembled and analysed. Integration of genome and transcriptome data was performed using two methods. Firstly, by Spearman correlation with a meta-analysis by combining gene level correlation coefficients from each study using a random effects model, weighting each study with inverse variance. Secondly using ANOVA, followed by combining gene level p-values across studies using Stouffers Z-score. Disease specific survival was examined at an individual gene level and using all genes simultaneously (via a Cox Boost analysis) to identify gene expression changes that are associated with poor outcome.

Results: DNA copy number profiling identified recurrent gains (1q, 8q, 16p), losses (11q, 16q) and amplifications (1q32, 8p12-p11.2, 11q13). 11q13 amplifications were prevalent at a higher frequency in patients with poor outcome compared to patients with better outcome. The integrative analysis identified 1928 candidate genes whose expression was associated with gene copy number; as expected being enriched from genome regions highlighted above. One hundred and sixty of these genes were of prognostic significance in the METABRIC ILC cohort, several are known cancer drivers (e.g. *PBX1*, *CCND1*) and five new candidates are being investigated as novel prognostic biomarkers by immunohistochemistry.

Conclusions: An integrated molecular analysis of ILC has identified a large number of gene expression changes that are dictated by gene copy number in ILC. Some of these are expected to influence tumour behavior and to highlight aggressive cancers with poor prognosis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-17

Title: Interrogating the impact of pregnancy on breast cancer biology using DNA copy number profiling

Nguyen B, Brown DN N, Rothé F, Desmedt C, Majjaj S, Pruneri G, Peccatori F, Azim Jr HA A and Sotiriou C. Jules Bordet Institute, Brussels, Belgium and European Institute of Oncology, Milan, Italy.

Body: Background

Epidemiological evidence indicates a clear relationship between pregnancy and breast cancer (BC) risk. However, little is known regarding the impact of pregnancy on BC biology. DNA copy number aberrations (CNAs) play an important role in breast carcinogenesis. BC during pregnancy is a rare disease but yet could serve as a good model to study the impact of pregnancy on BC biology.

Methods

We retrospectively included 54 pregnant and 113 non-pregnant BC patients matched for age and stage with complete clinico-pathological, gene expression and 5-year follow-up data. CNAs were assessed using Affymetrix OncoScan FFPE arrays. We identified the CNAs associated with pregnancy using a multivariate logistic regression adjusted for classical clinico-pathological features. We further evaluated their impact on gene expression.

Results

After quality control, CNA profiles were obtained for 38 pregnant and 87 non-pregnant BC patients. We identified 13 regions with copy number gains, 11 of which were more frequently gained in pregnant compared to non-pregnant controls and 5 regions with copy number loss, 3 of which were more frequently lost in pregnant patients ($p \leq 0.05$). Of interest, we identified 4 genes previously identified as driver event associated with CNAs in breast cancer (S. Nik-Zainal et al, Nature 2016). *AKT1* and *CDKN2A/B* were more frequently gained in the pregnant compared to the non-pregnant (23.7% vs. 8.0%, $p=0.068$ and 18.4% vs. 4.6% $p=0.036$) and *ARID1B* was less frequently gained in the pregnant cohort (2.6% vs. 13.8%, $p=0.02$). Interestingly, *PAPPA* which had been previously identified as a pregnancy-dependent oncogene (Takabatake Y. et al, EMBO Mol Med. 2016) was also more frequently gained in the pregnant compared to the non-pregnant patients (21.1% vs 5.8%, $p=0.03$). We next evaluated the effect of these CNAs on their own gene expression levels and found that *AKT1* and *CDKN2A/B* CNAs were affected by gene-dosage effect.

Conclusions

In this study, we were able to identify several genomic alterations associated with pregnancy that could further elucidate the impact of pregnancy on BC risk. Moreover, by combining CNAs with gene expression, we were able to identify genes whose expression were associated with CNAs and therefore could be considered potential drivers of this rare disease.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-18

Title: Genomic copy number alterations (CNA) associated with pCR in HER2-positive (HER2+) early-stage breast cancer (BrCa) patients receiving neoadjuvant trastuzumab (T)

Walsh N, Gullo G, Maguire A, O'Donovan N, Quinn C and Crown J. National Institute for Cellular Biotechnology, Dublin City University, Dublin, Ireland and St. Vincent's University Hospital, Dublin, Ireland.

Body: Introduction: Genetic alterations such as amplifications and deletions frequently contribute to tumorigenesis. These alterations can change gene expression which alters the normal cell growth and survival regulatory mechanisms. Characterisation of DNA copy number alterations (CNA) is important to understand cancer progression and response to therapy. The aim of this study is to determine patterns of CNAs in HER2+ early-stage BrCa patients achieving pathological complete response (pCR) to neoadjuvant T therapy.

Methods: Retrospective analysis of our database of 95 HER2+ BrCa (stages I-III) who received T neoadjuvantly revealed 46 % (44/95) achieved pCR compared with 53 % (51/95) who did not respond (NR). DNA from pre-treatment tumour biopsy specimens from neoadjuvant T therapy patients was extracted, and array-based comparative genomic hybridization (aCGH, n = 8; 6 pCR:2 NR) was used to identify CNAs, which correlated with pCR. Pathway analysis was then used to identify functionally relevant genes in aberrant regions.

Results: aCGH analysis of DNA from pCR and NR identified distinct patterns of CNAs. HER2 amplicon was confirmed by IHC and aCGH in all samples. Although there was no significant difference in the average CNAs between groups (20 ± 17 vs 17 ± 2), there was greater variation in the range of CNAs in pCR (8-56 CNA) compared to NR (15-19 CNA). More gains and amplifications were observed in pCR patients with more deletions in the NR group. The most common chromosomal amplification region included chr8q12.1-q24 with 87.5% of all cases displaying gains. Of the 6 patients who achieved pCR, 50% displayed a deletion in chr9 spanning p24.3-p21.3, consistent with a deletion of tumour suppressor CDKN2A. No aberrations in chr9 were observed in NR cohort. The deleted genomic region contained 65 common protein-coding genes, with the interferon biological pathway as the most significant ($p=1.03E-36$).

Conclusions: Distinct genomic CNAs were observed between patients achieving pCR compared to NR. However, of the 8 pts characterised here, none have relapsed. Follow-up data revealed a relapse rate of 6.8 % (3/44) vs 11.8 % (6/51) in the pCR and NR groups, respectively. To further elucidate the immunological response, we will present CNA data patterns on relapse and response and compare the impact of CNAs, immune-related proteins and pCR as surrogate predictors for outcome.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-19

Title: Homologous recombination deficiency in PD-L1, PD-L2, Jak2 (PDJ) amplified triple negative breast carcinoma

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Body: Introduction: Homologous recombination (HR) is essential for repairing double-stranded DNA breaks. Tumors deficient in DNA HR display high levels of copy number aberrations including amplifications, deletions, and breakpoints. Studies in BRCA^{mut} cancers have shown that HR deficiency (HRD) renders triple negative breast carcinoma (TNBC) sensitive to carboplatin or Poly(ADP-ribose) polymerase (PARP) inhibitors via a synthetic lethal interaction, as previously described. Clinical observations in breast and other solid tumors suggest that the HRD phenotype may arise independently of BRCA1/2 mutation. The implications of HRD enrichment in PD-L1, PD-L2, Jak2 (PDJ) 9p24.1 amplified TNBC are not known. Our goal was to determine the HRD status in PDJ enriched tumors, and the potential association of the PDJ amplicon with a BRCA like HRD phenotype.

Methods: Archival fresh frozen and formalin-fixed paraffin embedded (FFPE) tumor samples from 41 patients with TNBC were obtained from 1998-2015. Tumor populations (diploid, tetraploid, and aneuploidy) were sorted using DNA content flow cytometry and interrogated with oligonucleotide array comparative genomic hybridization (aCGH). Tumor homologous recombination deficiency (HRD) scores were calculated by number of interstitial aberrations (IA) in their genomes (HRD-IA). A cut-off of < 40 (low), ≥ 40 (high) or ≥ 50 (very high) was assigned based on our studies of known BRCA^{mut} tumors. Data were compared between HRD groups using chi-square, ANOVA F-test or log-rank test.

Results: 14/41 (34%) TNBC patients had high HRD-IA score and 6/41 (14.6%) had very high HRD-IA score. The median age was 61.8 years (SD = 12.8), the average tumor size was 3.1 cm (SD = 2.6), 42% were node positive, 24% were pre-menopausal, 19% were Black, Hispanic, or Asian/Pacific Islander. Genetic testing was negative for BRCA1/2 mutations in 9/41 patients, including 3 high and very high HRD-IA score patients. There was no difference in the OS at 5-years [74% versus 59% (p = 0.907)] in the high HRD-IA group. 12/41 (29%) patients were PDJ+. In the PDJ+ patients, 4/12 (33.3%) had HRD ≥ 40 and 1/12 (8.3%) had HRD ≥ 50 compared to the PDJ- patients where 10/29 (34%) had high HRD-IA score and 5/29 (17%) had a very high HRD-IA score, p=0.463. Patients with inflammatory TNBC were significantly more likely to have high HRD-IA score 4/6 (66%) compared to non-inflammatory TNBC 8/31 (26%), p=0.05.

Conclusions: We describe a unique group of TNBC patients with similar patient characteristics, tumor size, nodal status, and stage when compared by low, high and very high HRD-IA score. For inflammatory TNBC, there was statistically significant percentage of patients with a high HRD-IA score. We did not see a statistically significant correlation with 9p24.1 amplification based on HRD-IA score which has been suggested in other data sets. The HRD-IA score did not statistically impact OS but there was a suggestion that those with a higher score may have an improved OS potentially due to chemotherapy sensitivity. Further study with a greater number of patients to adequately power the study is needed to determine whether our initial findings can be confirmed and whether the HRD-IA score can be used to define a specific subset of TNBC patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-20

Title: Comparing the frequency and types of genetic aberrations between older and younger women with metastatic breast cancer at the University of North Carolina at Chapel Hill

Jolly TA A, Grilley-Olson JE E, Deal AM M, Ivanova A, Hayward MC C, Benbow JM M, Parker JS S, Patel NM M, Eberhard DA A, Weck KE E, Mieczkowski P, Dees EC C, Muss HD D, Reeder-Hayes KE E, Earp HS, Sharpless NE E, Carey LA A, Hayes DN and Anders CK K. University of North Carolina at Chapel Hill, Chapel Hill, NC and Genomic Health Inc., Redwood City, CA.

Body: Background: Targeted therapies have the potential to revolutionize cancer treatment in older adults as they are often oral, convenient, may be better tolerated than cytotoxic chemotherapy, and can be tailored to an individual's biomarker profile. We explore the frequency and distribution of potentially actionable genomic alterations among older (≥ 65) and younger (< 65) patients (pts) with metastatic breast cancer (MBC).

Method: Next generation genetic sequencing (UNCseq™) of a dynamic panel of target genes was prospectively offered to pts with MBC treated at the University of North Carolina at Chapel Hill (UNC). DNA libraries were prepared separately from a retrieved archival FFPE tumor sample and a matched normal sample from each pt. Relevant targets were enriched by custom Agilent SureSelect hybrid capture baits using standard protocols. Samples were sequenced on Illumina HiSeq 2000/2500 platforms. Mutational findings were reviewed by a molecular tumor board variants identified to be potentially actionable underwent confirmatory testing in a CLIA approved laboratory. Confirmed findings were inserted into the pt's EMR accessible by both the pt and the treating oncologist. Two-sided Fisher's exact test was used to compare percentages between age-specific groups.

Results: As of 3/31/16, results were available for 140 pts. 19% were 65 years or older. Breast cancer clinical subtypes were: HR+/HER2- 49%, HER2+ (HR any) 17%, TN 34% and metastatic location was: bone only 5%, visceral only 44%, bone & visceral 51%; no significant differences were observed between older and younger age groups. Older pts were more likely to be Caucasian compared to younger patients (92% v 75%, $p=0.06$). Overall, older patients had a higher total number of mutations compared to younger patients (see Table) ($p=0.04$). Mutation types were similar between age groups, although a trend for more PIK3CA mutations among older patients was seen (37% v 20%, $p=0.07$).

Observed Mutations by Age.

	≥ 65 years (%) N=27	< 65 years (%) N=113	p
Number of Mutations			
0	11	27	.04
1	48	49	.04
2	33	20	.04
3	7	4	.04
Type of mutation			
PIK3CA	37	20	.07
CCND1	7	9	.99
NF-1	11	5	.37
FGFR1	4	4	.99
PTEN	4	9	.69
EGFR	0	4	.99

Conclusion: Genomic alterations may allow therapeutic tailoring in both older and younger patients with breast cancer. In this

cohort with metastatic disease, older patients had significantly more mutations, but no clear difference in mutational types was seen by age. The relative small number of older pts in this cohort limits generalization, but supports the need for more extensive characterization of molecular aberrations among older pts with metastatic breast cancer in the new era of targeted therapy. Research support by the University Cancer Research Fund, NCI Breast Cancer SPORE grant (CA58223), John A. Hartford Foundation and Susan G. Komen Foundation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-21

Title: A comparison of oncotype DX recurrence scores in a screen detected vs a symptomatic cohort of patients with breast cancer: A UK experience

Khawaja S, Parab A, Thomas D, Huws A, Munir A, Udayasankar S, Sharaiha Y and Holt S. Prince Philip Hospital, Llanelli, United Kingdom.

Body: Background:

In the Western World, it has been stated that breast cancers detected on a screening program are indolent. There have been many recent publications stating that breast screening is overdiagnosing and therefore overtreating patients with breast cancer. With the advent of genomic testing, it can now be determined which patients have an aggressive tumor requiring systemic chemotherapy. We therefore conducted a retrospective study in the UK on patients having oncotype DX testing in both screen detected and symptomatic cancers.

Materials and Methods:

Patients in our institution undergoing oncotype DX testing for invasive breast cancer which was ER positive and node negative were part of this study. The detection of the breast cancer was documented as either a screening case or a symptomatic one. The recurrence scores of the oncotype DX testing was then compared in the screening versus the symptomatic cohort.

Results:

155 patients were included in this study. They underwent Oncotype DX testing between 2008 to 2016. The age of the patients ranged from between 31 years to 78 years. Eighty-nine patients were reported to have a low recurrence score; 45 had an intermediate score; and 21 had a high result. Fifty eight patients were screen detected, while 97 patients were symptomatic presentations. In the screening population, 32 patients had a low recurrence score; 22 had an intermediate result and 4 had a high recurrence score resulting in the latter groups being considered for chemotherapy. In the symptomatic cohort, 57 had a low recurrence score; 23 had an intermediate result; and 17 had a high score.

Conclusion:

The results of our study depict that even patients in a screening cohort will have a high number of intermediate recurrence scores and some with a high recurrence score. This shows that the hypothesis that screening detects a majority of breast cancers which are indolent not requiring further systemic treatment should be looked at again in light of our results with genomic testing.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-22

Title: The value of RNA-Seq for the detection of clinically actionable targets in breast cancer - A small cohort analysis

Meissner T, Amalraja A, Mark A, Andrews A, Connolly C, Young B, De P, Williams C and Leyland-Jones B. Avera Cancer Institute, La Jolla, CA and Avera Cancer Institute, Sioux Falls, SD.

Body: Introduction

Next generation sequencing has facilitated the understanding of pathogenesis and molecular heterogeneity of breast cancer (BC) as well as accelerated the path towards precision medicine. DNA sequencing (DNA-Seq) based assays for the detection of mutations and alterations in solid and hematologic cancers are finding their way into clinical practice and are readily available as clinical products. RNA sequencing (RNA-Seq), so far being vastly applied in the research context, promises to expand the diagnostic, prognostic and therapeutic use of this technology in cancer. Beyond mutational status, RNA-Seq enables the detection of fusions, quantification of gene expression level, detection of differentially expressed genes, molecular based subtyping, and risk-stratification. In this study we analyzed RNA-Seq and copy number data from BC patients that had undergone DNA-Seq based diagnostics through commercial providers with the goal to detect additional actionable targets.

Materials and Methods

We included 18 BC patients (5/18 triple negative) that had previously undergone DNA-based targeted (321 genes) sequencing. RNA-Seq to a minimum of 75M reads (75pb) was performed using 100 ng of total RNA on the Illumina NextSeq 500 platform. STAR was used for alignment (hg19) and gene expression quantification (RefSeq). Fusions were detected using STAR-Fusion. DESeq2 was utilized to identify patient specific differentially expressed genes by analyzing samples individually against a set of 13 controls from healthy breast tissue generated in-house. Copy number variations (CNVs) were detected using the Nanostring CNV Cancer panel (89 genes) on the Nanostring nCounter platform. Differentially upregulated or amplified genes were queried against DGIdb and Gene Drug Knowledge database for suitable drug matches, limiting the queries to clinically actionable antineoplastic drugs.

Results

Analyzing the cohort of 18 BC patients, we detected on average 26 BC relevant genes (526 total, \log_2 FC > 2) to be upregulated per patient. Querying the upregulated genes against DGIdb, we found a total of 18 genes that had drug matches and fulfilled the criteria of being actionable antineoplastic drugs, with 17/18 samples having a minimum of two gene targets (avg: 4). Most frequent upregulated genes were TOP2A (83%), AURKA (61%), AURKB (56%), RET (39%) and FGFR3 (28%). In the case of CNVs, 12/18 patients showed at least one gene target with clinically actionable drugs associated. This was observed across 12 gene targets that were amplified (avg: 3) and 4 gene targets that underwent deletions (avg: 1). Most frequent CNVs included MYC (14%) and CCND1 (12%). 4/7 patients having an AURKA overexpression also showed an AURKA amplification on the CNV assay. 10/18 patients had fusions events, with an average of three fusions per patient, including GAB2-WNT11, PAK1-TENM4 and FGFR2-CEP55 fusions.

Conclusions

We show that RNA-Seq and copy number assays provide additional clinical value by detecting suitable drug targets beyond traditional DNA-based approaches. We are conducting further analysis on how these additionally derived drug targets could improve the current treatment schedule of those patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-23

Title: Utilities and challenges of RNA-Seq based expression and variant calling in a clinical setting

Young B, Mark A, Meissner T, Amallraja A, Andrews A, Connolly C, Williams C and Leyland-Jones B. Avera Cancer Institute, La Jolla, CA and Avera Cancer Institute, Sioux Falls, SD.

Body: Introduction

Variant calling based on DNA samples has been the gold standard of clinical testing since the advent of Sanger sequencing. The use of DNA variants has proved a great value to guide treatment in cancer patients. However, DNA based analysis will not inform about expression status of the gene harboring a particular variant. RNA has long been used to monitor expression. To this point RNA assays and analysis are confined to the research laboratory and rarely used clinically except in specifically defined gene signatures such as PAM50 and OncoType Dx. Beyond expression, RNA has the ability to confirm expression of DNA variants and identify fusion events. We hypothesize that the combination of DNA and RNA based data will allow the determination of variant specific expression status and improve clinical diagnostics. It has been previously shown that RNA sequencing (RNA-Seq) based variant calls are highly accurate and confirm DNA based variant calls. In this study we investigated the utility of RNA-Seq as a diagnostic assay integrated with DNA based sequencing data.

Materials and Methods

Targeted DNA sequencing of 321 genes was performed on 37 patient samples (FFPE), including 22 breast cancer samples by a commercial vendor. RNA-Seq on the same patient samples was performed using 100ng of total RNA. Libraries were run on the Illumina NextSeq 500 with a minimum of 75M paired 75bp reads. To evaluate RNA-seq expression reproducibility, replicates of 6 normal ovarian tissue samples (min. 50M reads) were run in sets of triplicates. STAR was used for alignment (hg19) and gene expression quantification (RefSeq). RNA-Seq based variant calling was performed using the SNPiR pipeline. Based on the results of the commercial assay, DNA based variants were examined for expression of the corresponding genes and ability to confirm variants in the RNA-Seq data.

Results

RNA expression data showed no corresponding gene expression for at least one single nucleotide variant (SNV) in 9/37 patients analyzed (24.3%). In 18/37 patients (48.6%) SNV corresponding expression was in the lowest quartile of expression values. Variant calls could be confirmed by RNA-Seq for 95/455 SNVs, with adequate coverage in 263 of the remaining 360 variant locations (median coverage: 34). Of these, a homozygous reference call was made in 166/263 SNVs. Concordance for RNA-Seq gene level expression data between replicates was > 0.995 .

Conclusions

These findings suggest that RNA-Seq based data can provide clinical value when using gene expression values in combination with DNA based variant calls. We found gene level expression to be highly reproducible and will further investigate the use of spike in controls to determine clinically usable expression ranges and lower limit of expression values. To our knowledge, it has not been shown that RNA-Seq based variant calls are reproducible which is the focus of our current research as this will be one requirement for usage in a regulated environment. While our use of RNA Seq is currently limited to gene expression level data, we have demonstrated a clinically relevant benefit to using RNA Seq data as an additive feature to the current standard of DNA variant calling.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-24

Title: A head-to-head comparison of paraffin-embedded MammaPrint versus oncotype DX: A McGill University Health Center experience

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Body: Background: Breast cancer is the most common malignancy among women. With the introduction of improved imaging techniques and the general population's awareness of breast cancer, increasing numbers of patients are more frequently diagnosed at a very early stage. The Oncotype DX Recurrence Score (ODX) is commonly used to estimate recurrence risk and chemotherapy benefit in ER positive, node negative breast cancer. Earlier studies showed that women with Oncotype DX Recurrence score results of 11 or less had excellent outcomes which proved that thousands of patients worldwide can forgo chemotherapy and its harmful side effects based on a low Recurrence score. Now another test called the MammaPrint has been approved by the United States (US) Food and Drug Administration to assess the risk of cancer relapse. The primary study objective was to evaluate the concordance of patient results with Oncotype Dx (ODX) when compared to the 70-gene signature MammaPrint (MP), the 80-gene signature of Blueprint (BP) and TargetPrint (TP)

Methods: This retrospective clinical study was carried out in the Oncology Department at the Royal Victoria Hospital (RVH), part of the MUHC. Eighty-six consecutive patient-slides node negative hormone positive breast cancer tissue tested with the Oncotype DX between October 2008 and September 2014 were included. Further inclusion criteria were age > 18. These same slides were then analyzed and classified with MammaPrint as low or high risk by Agendia Laboratories. Blueprint (BP) and TargetPrint (TP) analysis were also performed by Agendia Laboratories.

Results: Of the 16 ODX RS between 0 and 11, 13 were low risk by MP (81 % agreement) and 16 were low risk by BP (100% agreement). Of the 50 ODX low RS cases, 33 were low risk by MP (66 % agreement) and of the 9 ODX high RS, 7 were high risk by MP (78 % agreement). Of ODX intermediate risk cases (27), 14 of were MP low risk (52 %), (48 %) 13 were MP high risk. Of BP low risk luminal tumors, 33/49 (67%) were ODX low, 14/49 (29%) ODX intermediate, and 2/49 (4%) ODX high risk. BP class was correlated with ER, PR and HER2 results. Overall agreement between clinical ER, PR, HER2 (IHC+FISH) results with TP results were 98% (81/83), 83% (69/83), 99% (82/83), and percent positive agreement for HER2 was 0/1 (of unequivocally HER2 positive cases identified correctly by TP).

Conclusion:

Our results show that there is a high concordance between ODX RS of 11 or less and low risk MammaPrint and Blueprint scores. When the ODX RS is above 11, our study shows that there are real differences in risk stratification between MP and ODX. At this point, there is no consensus seen when Oncotype DX is above 11.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-25

Title: Patterns of translational dysregulation in models of estrogen receptor(+) and (-) breast cancer

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Body: Although our understanding of transcriptional dysregulation in cancer has expanded dramatically over the recent years, comparatively little is known about dysregulation in gene expression at the translational level. Yet, gene expression is extensively modulated at the level of translation. Leveraging the unbiased nature of next generation sequencing, we utilized the ribosome profiling strategy to characterize the translational profiles of a representative ER(+) (T47D) and ER(-) (SUM159) breast cancer cell line and compared them with the profiles of non-malignant human mammary epithelial cells (HMECs). The overarching hypothesis is that cancer cells differ from their non-malignant counterparts not only at the level of transcription but also at the level of translation and coordinate changes in transcription and translation lead to the phenotypic hallmarks of cancer.

The results show that the median translational efficiency was higher and more variable in malignant cells as compared to HMECs (HMECs 1.09; T47D 1.6; SUM159 1.29. Variance of the log2 transformed values, 2.37; 3.92; 4.16, respectively). When cells were deprived of serum for 24 hours, translation was uniformly upregulated but this upregulation was more pronounced in malignant cells. A larger number of genes were differentially expressed at the translational as compared to the transcriptional level between non-malignant and malignant cells. There was an imperfect correlation between RNA levels and translational efficiency. In addition, individual transcripts were translated with variable efficiency between malignant and non-malignant cells suggesting that the translation of the same RNA sequence is modulated in a context-dependent manner.

Although translation is globally upregulated in malignant cells, this upregulation is not uniform and the translational efficiency of multiple cancer-related mRNAs is not proportional to their abundance. For example, while the abundance of the *CDK1* mRNA was not significantly different among the 3 cell lines, its translational efficiency was 1.5 and 2 times higher in the SUM159 and T47D cells, respectively. On the other hand, the mRNA of its cognate inhibitor *CDKN1A* was not only 4 times less abundant in the malignant cells but also half as efficiently translated as compared to HMECs.

Ribosome profiling has consistently revealed translation outside of canonical protein-coding regions. While in HMECs only 5.3% of unique ribosome protected fragments aligned to non-protein encoding transcripts, the respective fractions were 8% for T47D and 19.2% for SUM159 cells and were consistently higher in malignant cells across all conditions tested. We found that the translation of RNAs previously thought to be noncoding, especially lincRNAs, was pervasively upregulated and to similar levels in T47D and SUM159 cells as compared to HMECs.

Collectively, these results begin to unravel components of dysregulation in gene expression at the level of translation with important implications for the molecular pathophysiology of breast cancer and set the stage to advance such studies in primary human tumor samples.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-26

Title: Genomic analysis of molecular discordance of paired primary and recurrent triple negative breast cancer

Yuan Y, Li A, Yost S, Yuan Y-C, Chu P, Warden C, Wang J, Liu Z, Liang Y, Goldstein L and Wu X. City of Hope Comprehensive Cancer Center.

Body: Background: Triple negative breast cancer (TNBC) is a heterogeneous disease with several molecular subtypes: basal-like1 (BL1), basal-like 2 (BL-2), mesenchymal(M), mesenchymal-stem-like(MSL), immune-modulatory(IM) and unclassified (UNC) Molecular evolution of TNBC through chemotherapy selection pressure is well recognized but poorly understood. This study was carried out to perform paired genomic analysis of TNBC comparing primary breast cancer with recurrent/refractory disease. Here we report the result of the first10 paired tissue analysis.

Methods: 49 paired specimens were identified through an IRB-approved protocol via COH biorepository search (2002- 2015). miRNA and mRNA profiling of 22 samples were performed. The miRNA libraries were prepared and sequenced on Hiseq2500. Sequences were aligned to hg19 genome and miRNA expression levels were counted by in house built R scripts. GO and pathway annotation was performed using DAVID online tool. Affymetrix human Genechip 2.0st was used for mRNA expression profiling. Raw data were normalized and processed using Expression Console, and linear regression was performed using Limma to identify the differentially expressed genes between primary and recurrent/refractory TNBCs.

Result: Through mRNA profiling, we identified several unique gene expression patterns comparing the paired TNBC. Significant mRNA expression alterations were observed in: cell cycle, DNA repair and adhesion. Using Vanderbilt TNBC sub-classification tool, we have identified "phenotype shift" between primary and recurrent TNBCs. Of the 8 paired specimen analyzed, 3 paired tissue remain in the same subclass (2 in IM, 1 in M). Phenotype shift observed in: 1 from BL1 to BL2, 1 from BL2 to BL1, 1 UNC to IM; 1 MSL to UNC; 1 from M to UNC. 15 up regulated and 13 down regulated miRNAs were identified. Most significantly differentially expressed miRNA (with more than 4 fold expression changes, P-value < 0.001) included: miR-206, miR-203, miR-144, miR-16-2, miR-15b, and miR-20b (un-regulated) and miR-10b, miR-125b and let-7c(down-regulated). These miRNA genes are involved in regulation of hormonal receptor signaling, cell cycle, proliferation and metastases. Statistically significant differentially expressed miRNAs identified from our TNBC patient cohort will be further validated using RT-PCR.

Conclusion: A number of mRNA gene pathways and miRNAs showed differential expression between paired recurrent and primary TNBC tumor specimen. The underlying biology driven the phenotype shift is being studied. Further analysis to include a total of 49 paired TNBCs is currently underway.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-27

Title: Evaluation of the Oncomine focus and comprehensive assays for therapeutic stratification in early hormone receptor positive breast cancers

Bayani J, Crozier C, Zhang NX, Amemiya Y, Quintayo MA, Yan FJ J, Dion D, McCormack S, Yaffe M, Seth A, Feilotter H and Bartlett JMS MS. Ontario Institute for Cancer Research, Toronto, ON, Canada; Sunnybrook Health Sciences Centre, Toronto, ON, Canada; Queen's University, Kingston, ON, Canada and University of Toronto, Toronto, ON, Canada.

Body: Large-scale sequencing initiatives have revealed a wealth of common and novel variants as well as copy-number aberrations, across different solid tumours and hematological malignancies. The growing list of variants/aberrations can sometimes be matched to specific therapeutics. Such “actionable mutations/changes” hold promise for personalized treatment, as treatments could be tailored to molecular abnormalities, rather than disease site. In breast cancer, women with hormone positive early breast cancer continue to experience improved survival on adjuvant anti-hormone therapy, but even today, a significant number of women continue to progress. Therefore there is not only a need to identify those women for whom current therapies are insufficient, but to identify alternative therapeutic interventions. The ThermoFisher Scientific Oncomine™ Focus and Oncomine™ Comprehensive Assays (OFA and OCA) are based on the Ion Torrent™ next-generation sequencing platform and Ion AmpliSeq™ library preparation technology, coupled to the Oncomine™ Knowledgebase, for target selection, variant calling, and data annotations. Both panels interrogate the most referenced oncology biomarker variants that are matched to curated published evidence from clinical trials supporting the matching of driver genetic variants with relevant potential clinical therapeutic options. The ability to identify SNVs, CNVs and fusion events in a single assay provides an unprecedented approach to maximizing the molecular information to be derived from a single tumour sample. To explore the value of the Oncomine™ assays in early invasive breast cancers, we have performed a pilot study to assess the reproducibility and accuracy of the OFA and OCA from nucleic acids extracted from formalin-fixed paraffin embedded tissues. In addition to the sequencing and copy-number data generated by these assays, we will compare these results to copy-number information generated using the Oncoscan® (Affymetrix) copy-number assay as well as information derived by Multiplex Ligation-dependent Probe Amplification-based panels (MRC-Holland) and Fluorescent *in situ* Hybridization (FISH). Our preliminary analyses of 35 invasive breast cancers by Oncoscan® identified the frequent whole chromosomal gains of 2, 3, 5, 7, 18, 19 and 20; gains of 1q, 7p, 8q, 11p, 16p, 17q; losses at 1p, 8p, 11q, 13, 16q, 17p and chromosome 18. High level amplifications were also identified for breast cancer related genes such as ERBB2, CCND1, MYC, FGFR1; in addition to the frequent losses of TP53, RB1, CDKN2A. Copy-number changes were confirmed by locus-specific FISH and MLPA. Data generated from the OFA and OCA from these same samples will be compared to the other platform findings and provide a snapshot of the mutational landscape of early breast cancers across these pan-cancer panels. Having established the robustness and accuracy of the assays, the applicability of the OCA in the context of improved stratification for breast cancers for prognostic and predictive tests will be discussed.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-28

Title: Melatonin treatment: A transcriptomic networks in a xenograft model of breast cancer

Jardim-Perassi BV V, Sonehara NM M, de Paula-Junior R, Chammas R, Coutinho LL L, Reis Júnior O, Alexandre PP P, Fukumasu H and Zuccari DAPC APC. Faculdade de Medicina de São José do Rio Preto, Laboratório de Investigação Molecular do Câncer, São José do Rio Preto, Sao Paulo, Brazil; Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, Sao Paulo, Brazil; Universidade Estadual de Campinas, Instituto de Biologia, Campinas, Sao Paulo, Brazil and Universidade de São Paulo, Faculdade de Zootecnia e Engenharia de Alimentos, Pirassununga, Sao Paulo, Brazil.

Body: Melatonin is a hormone produced by the pineal gland and has been shown different antitumor effects, as immunomodulatory, antioxidant, pro-apoptotic, anti-proliferative, antimetastatic and antiangiogenic, however, the pathways by which melatonin exerts its action need to be identified. Thus, the aims of this study were to perform the transcriptome analysis to evaluate the pathways of melatonin action in triple-negative breast cancer. Triple-negative breast cancer cells (MDA-MB-231) were injected into the mammary gland of the athymic nude mice (n=10), which were treated with melatonin (40 mg/kg) or vehicle during 21 days. RNA-Seq libraries were created using Truseq RNA-Seq Library Prep Kit v2. The experiment was paired-end with 100nt read length, performed on the Illumina HiSeq2500 sequencer, producing about 30 million reads per library. To differentiate human and mouse expression, the alignment was performed to filter out mouse-like reads before mapping to the human reference and vice versa, and data were mapped against human (GRCh37/hg19) and mouse (NCBI37/mm9) genomes separately, using the TopHat software. The HTSeq was employed for analyses of read counts and DESeq2 was used to identify genes differentially expressed between melatonin treated and control tumors. Differentially expressed genes (DEGs) were identified based on a false discovery rate (FDR) q -value threshold of less than 0.05. Also, we applied Weighted Gene Co-expression Network Analysis (WGCNA) to detect clusters of highly co-expressed genes (modules). Results showed that animals treated with melatonin had smaller tumors volume than controls ($p < 0.05$). RNA-Seq data showed that 57.24% of reads mapped uniquely to human, 29.66% reads in mouse and 11% reads mapping to both human and mouse genomes. In human tumor cells, there was no DEGs between melatonin treated and control group ($adjP > 0.05$). In mouse cells, which represent the tumor microenvironment, there were 34 DEGs between animals treated with melatonin and controls ($adjP < 0.05$). In tumors cells, we detected 714 differentially co-expressed genes ($|K_{diff}| > 0.6$), which were functionally enriched for GO terms like lipid metabolic process, response to drug, oxidoreductase activity and PPAR signaling ($adjP < 0.1$). Also, we identified 3 gene modules strongly associated with melatonin treatment, which were related with metabolic pathways ($adjP < 0.1$). In mouse cells, were detected 1345 differentially co-expressed genes, which were enriched for signaling pathways like Wnt receptor, Hedgehog and TGF-beta ($adjP < 0.1$). There were 3 gene modules strongly associated with melatonin treatment, which are enriched for regulation of translation and cell cycle, immune system process and T cell differentiation, regulation of action cytoskeleton and ErbB signaling pathway ($adjP < 0.1$). Also, potential regulator genes for melatonin treatment were detected by generating clusters of co-expressed genes and individual analysis confirms these results. Transcriptomic network analysis coupled with other results showed that melatonin treatment controls the tumor growth, acting especially by metabolic pathways in tumor cells and modulating the tumor microenvironment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-29

Title: Genomic variants in 5 male breast cancers using whole exome sequencing

Agrawal V, Zhong M, Huang W, Kewarpu P, Dimitrova N and Fallon JT T. Philips Research North America; New York Medical College/Westchester Medical Centre and Philips Healthcare.

Body: Breast cancer in men is a rare disease. Less than 1 percent of all invasive breast cancers occur in men and the lifetime risk of men being diagnosed with breast cancer is 1 in 1000; very low compared to in 1 in 8 for women. Due to the absence of an elaborate lobular system in the male breasts, most male breast cancers tend to be ductal and invasive. Paget's disease, inflammatory breast cancer and ductal carcinoma in situ are the other relatively rare breast cancer types in males. While there are similarities between breast cancers in both genders, there are also significant difference between the two cancers in terms of demographics, progression etc.

In order to assess the genes and pathways effected in male breast cancer, we performed whole exome sequencing of tumors from 5 patients (all aged above 60 years) with male breast cancer (MBC). All tumors were ER, PR positive and Her 2 Negative by IHC. For the five patient tumors, we identified an average of 47,3804 variants, 63% of which were within coding regions, spanning 14,314 genes. All tumors carried a total of 14769 variants in common, of which ZNF717, CDC27 and mucin family genes were among the most polymorphic genes. A total of 33 premature stop variants were found common across all tumors. These included variants in genes such as ANAPC1 and CDC27 that are associated with APC/C, an important component for the ubiquitination and degradation of G1 and mitotic checkpoint regulators. CDC27, a core component of the anaphase-promoting complex (APC), is a cell cycle regulator, which participates in control of mitotic checkpoint and maintenance of chromosomal integrity. Aberrations in CDC27 are associated with disruption in the normal functioning of cell cycle. Members of the mucin family have been reported to promote tumorigenesis by multiple mechanisms including activation of ERBB2 gene, which makes these variants in the mucin family genes potential driver events. Identifying the transcriptional regulation targets of ZNF717 may explain the high frequency of variants in this gene in all MBC tumors.

Comparison of the common variants across all MBC patients with TCGA female breast cancers revealed 500 variants that were called as somatic mutations in TCGA female cohort. Of 200 genes that contained these 500 variants, we found enrichment of genes involved in cadherin signaling and WNT pathways, both of which are known to be involved in tumor progression. The finding of common gene mutations and pathways associated between male and female breast cancer suggest a common underlying mechanism driving both, male and female breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-30

Title: Genomic and microenvironmental intra-tumor heterogeneity in DCIS

Fortunato A, King L, Mallo D, Kovacheva V, Yuan Y, Boddy A, Graham T, Aktipis A, Mardis ER R, Hall A, Marks JR R, Hwang S and Maley CC C. Biodesign Center for Personalized Diagnostics, Arizona State University, Tempe, AZ; Duke University, Durham, NC; The Institute of Cancer Research, London, United Kingdom; Arizona State University, Tempe, AZ; Barts Cancer Institute, Queen Mary University of London, London, United Kingdom and McDonnell Genome Institute, Washington University, St. Louis, MO.

Body: Intra-tumor heterogeneity drives neoplastic progression by supplying the fuel for natural selection among neoplastic cells. It also complicates screening and treatment of neoplasms. We hypothesize that the degree of intra-tumor heterogeneity in DCIS should predict which tumors are likely to become invasive and metastatic. We initiated a pilot project to test this hypothesis by comparing 9 cases of pure DCIS to 9 cases of DCIS with adjacent invasive disease. For each case, we sequenced the exome from two spatially distinct regions of DCIS as well as normal tissue taken from a lymph node with no tumor involvement. This required the development of new methods to extract high quality sequencing data from small amounts of DNA extracted from FFPE samples. We calculated the genetic divergence between the two tumor regions, defined as percent of the sequenced regions of the genome showing differences between the two samples that had sufficient sequencing coverage and quality scores for confident scoring. We also employed automated imaging analysis to score microenvironmental differences between the two tumor regions. These microenvironmental measures are based on ecological methods for measuring organismal interactions and habitats. We will present initial data on differences in phenotypic and genotypic intra-tumor heterogeneity comparing pure DCIS to DCIS associated with invasive breast cancer. Our methods can be readily translated to large tissue banks of FFPE samples from DCIS.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-05-31

Title: Studying the interactome of breast cancer: The cancer cell map initiative

Kim M, Kim K, Soucheray M, Swaney D and Krogan N. University of California, San Francisco, San Francisco, CA.

Body: Recent progress in genome sequencing has revealed numerous mutations in cancer genomes. But how many of these alterations result in changes in normal cellular processes is poorly understood. In addition, the biological functions of the majority of genes (e.g. BRCA1/2) with cancer-associated mutations have not been fully characterized, despite their expression and/or activity being highly correlated with cancer. Through years of study, it is becoming clear that cancer is a disease that arises not only because of defects in individual genes and proteins, but also because of the action of hallmark cellular processes and biological pathways. Therefore, what is urgently needed is to put the cancer genomic information into biological context by mapping mutated genes onto the complexes and pathways in which they function. The goal of this study is to uncover the comprehensive protein-protein interaction networks and pathways in various breast cancer subtypes to better understand how mutated cancer genes and genomes hijack and re-wire pathways and complexes during the course of breast tumorigenesis. Here we catalog protein-protein interactions for more than 40 genes recurrently mutated in breast cancer, using affinity purification and mass spectrometry (AP-MS). To identify co-associated proteins, cDNA clones expressing each protein were tagged with 3xFLAG at either N or C-terminus and introduced into MCF10A (non-tumorigenic “healthy” control), MDA-MB-468 (basal-like), MDA-MB-231 (claudin-low) and MCF7 (luminal A subtype) cells using doxycycline-inducible lentiviral vectors. For proteins with prevalent mutations (e.g. PIK3CA-H1047R, AKT1-E17K), mutant cDNA clones were also analyzed in parallel. Our interaction network reveals subtype and mutation-specific protein-protein interactions, most of which are not previously reported. We anticipate the breast cancer interactome study will uncover many previously unidentified aberrant pathways and protein complexes uniquely operating in breast cancer cells, and thus pinpoint proteins central in these pathways and complexes that may potentially serve as distinct biomarkers or therapeutic targets for tumors having the same or similar subtypes and/or genomic mutations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-06-01

Title: Evaluation of subclonality in the CTC and DTC compartment of patients with metastatic breast cancer using low pass whole genome and AmpliSeq panel sequencing

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Body: Background

A growing understanding of the molecular biology of cancer and the identification of specific aberrations driving cancer evolution have led to the development of various targeted agents. Tumors can exhibit significant heterogeneity and this may change over time, also as the result of selective pressure. Circulating tumor cells (CTCs), shed from multiple tumor sites, have demonstrated to represent of the overall tumor burden. We report the results of an ongoing comparative study on mutation and copy number profiles of primary and metastatic tissue, CTCs, and synchronously isolated DTCs from metastatic effusions of patients with clinically progressive MBC.

Materials & Methods

CTCs and DTCs were enriched from 7.5 ml blood or effusion using the CellSearch system and were further purified and sorted with the DEPArray system. For this study we isolated both 70 single and 70 pools of 10-200 CTCs or DTCs, in order have enough power to detect 5% subclones and analyse heterogeneity. Single and pooled WBCs were isolated as technical controls. DNA was isolated and amplified using the Ampli1-kit and subjected to Illumina WGS and Ion Torrent AmpliSeq panel sequencing. Fresh frozen tissue from solid metastases and the primary tumor, and bulk CTC (CellSearch Profile) were sequenced as comparators for mutation and copy number profiles. DNA of buffy coat was sequenced to enable germline variant detection. For mutational analysis, only somatic variants with good quality metrics, >20x coverage, variant allele frequencies >10%, and being non-synonymous or splice site variants, were taken into account.

Results

AmpliSeq panel sequencing was performed on 153 unique samples of three patients with a mean coverage depth of 1000x. In patient 1, a *PIK3CA* hotspot mutation was found clonally in all tumor samples at heterozygous level. Furthermore, various private mutations were found in both CTCs and DTCs, however not in WBC, including several *TP53* hotspot mutations. In patient 2, another *PIK3CA* hotspot was present in all CTCs at heterozygous frequencies. In patient 3, an enormous heterogeneity was observed between all CTC and DTC samples. For patient 3, disease evolution was detected during multiple events of progressive disease over 2 years. At the moment, low pass WGS for CN detection for all samples is being performed and results will be present prior to the SABCS.

Conclusion

Based on the mutational status we conclude that both clonal mutations as well as various private variants are present in single and pools of CTCs and DTCs. In addition to the detection of targetable aberrations, the evaluation of heterogeneity is of clinical importance, as the effect of targeting subclones is currently being explored in clinical trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-06-02

Title: Impact of heterogeneity of DCIS on immune cell infiltrations

Badve S, Gökmen-Polar Y, Harris AL L, Sui Y, Sevinsky C, Santamaria-Pang A, Ginty F, Tan PH and Gerdes MJ J. Indiana University School of Medicine, Indianapolis, IN; University of Oxford, Oxford, United Kingdom; GE Global Research, Diagnostic Imaging and Biomedical Technologies, Niskayuna, NY and Singapore General Hospital, Singapore, Singapore.

Body: Background: Ductal carcinoma in situ (DCIS) accounts for at least 20% of breast cancers. Factors associated with recurrence of DCIS or progression to invasive carcinoma are not well delineated. The goals of the current study were to profile the epithelial and immune cells using the MultiOmyx hyperplexed immuno-fluorescent based analyses. This was coupled with semi-automated algorithms to characterize the inter-relationships between cell populations within individual DCIS lesions.

Patients and Methods: Analysis for 15 antibody markers (EGFR, Her2, Her4, S6, pMTOR, PCAD, CD44v6, NaKATPase, SLC7A5, CD4, CD8, CD20, CD68, and CD10) was performed on a single FFPE section containing 10-20 distinct ducts from 13 cases of DCIS. Briefly, approximately 40 fields of view (FOV) from digitized sections containing DCIS or normal tissue were sequentially (cyclically) stained for the 15 markers. Each cycle entailed staining with 2-3 markers followed by imaging, dye inactivation, and re-staining. DAPI was used for nuclear demarcation and for registration of the images, while S6, pan-cadherin, Na+K+ATPase and pan-cytokeratin were used for epithelial segmentation. K-means clustering was used to determine patterns of co-expression of markers at the single cell, duct, and patient levels. These clusters were then correlated with immune marker expression by tumor infiltrating lymphocytes (TILs) by marker type (CD4, CD8, and CD20) and tumor compartment (stromal versus intraepithelial).

Results: Analysis of the epithelial component in each of 13 cases of DCIS (n= 415 ducts) revealed 8 distinct expression patterns (clusters) using a panel of 7 markers (EGFR, Her2, Her4, pMTOR, CD44v6, SLC7A5, and CD10). The frequency and distribution of clusters, annotated at the single cell level, showed that 4 DCIS's were dominated (>80%) by a single cell phenotype represented by cluster groups 3 and 7 (high Her2), cluster 6 (High Her4 and SLC7A5 and low Her2), or cluster 4 (non-descript). In 5 pts, the pattern was more heterogeneous consisting of mixture of cell populations with 50-70% of the cells belonging to cluster 1 (moderate to high levels for all markers except EGFR and CD10). The remaining pts had a strong representation of cluster 4 and 5 (CD44v6 and phospho-mTOR) cells. The distribution of both intra-epithelial and stromal TILs in DCIS cases were either consisted of a mixed B-cell (CD20+) and T-cell response (n=4), or one dominated by T-cells. Cluster 2 (High EGFR and CD10) was associated with a largely T-cell response ($r_s = 0.83$, P value = 0.0004), while Cluster 7 (strong HER2) was associated with a B-cell response ($r_s = 0.68$, P value = 0.009).

Conclusions: Analysis 15 markers and use of K-means clustering algorithm, shows prominent inter-tumoral (but not intra-tumoral) heterogeneity in DCIS. Furthermore, epithelial cell specific clusters (high HER2 or EGFR) were associated with distinct B or T cell infiltration by TILs. Additional ongoing studies will determine the clinical significance of the clusters with respect to recurrence of DCIS and development of invasive carcinomas.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-06-03

Title: Serial evolution of hormone receptor status and mutational profile among patients with metastatic breast cancer

Henderson L, Brachtel E, Fitzgerald D, Gadd M, Specht M, Thabet A, Gurski J, Sgroi D, Moy B, Isakoff S, Bardia A and Juric D. Massachusetts General Hospital, Boston, MA.

Body: Introduction: Tumor heterogeneity presents a significant impediment to identifying appropriate treatments for patients. Genetic mutations and hormone receptors are frequently used as a guide for selecting appropriate targeted or hormonal therapies, however it is possible that these markers may change over time, leading to reduced effectiveness of these treatments. In this study, we review the results of serial and paired biopsies to identify receptor switch in estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status as well as to identify changes in clinically relevant mutations, including spatial and temporal heterogeneity.

Methods: We identified a total of 237 patients initially presenting with ER+/HER2 negative breast cancer and who had multiple biopsies during the course of their treatment, including at least one in the metastatic setting. ER, PR, and HER2 status for each of these serial biopsies was gathered from chart reviews. HER2 results by both IHC and FISH were collected. PIK3CA mutations were also assessed by Snapshot utilizing multiplexed PCR of common hotspot mutations using DNA derived from formalin-fixed, paraffin-embedded (FFPE) tissue.

Results: From a total of 213 patients with known ER status for multiple serial biopsies, we identified 9.4% (N=20) who had at least one change in ER status over time. From a total of 198 patients who had documented PR status for multiple biopsies, 40.4% (N=80) had at least one change in PR status. Changes in HER2 status were similarly assessed, with 6.7% of patients having at least one change by IHC and 4.4% of patients having at least one change by FISH. Of those patients exhibiting changes in ER status, 6 were noted to have multiple changes over time. Of those with changes in PR status, 18 had multiple changes over time. Changes in hormone receptor status were also noted to occur between serial biopsies in the metastatic setting. A total of 128 patients had ER results available for multiple metastatic specimens, of which 8.6% (N=11) had at least one change in ER status. A total of 116 patients had PR results available for multiple metastatic biopsies, of which 38.8% (N=45) had at least one change in PR status. Changes were also noted in the metastatic setting in HER2 (IHC) with a frequency of 8.7% and in HER2 (FISH) with a frequency of 4.7%. A subset of 108 patients were identified as harboring a mutation in PIK3CA. Within this population, 9.6% of patients had at least one change in ER status over time and 34.1% had at least one change in PR status. 9.0% exhibited at least one change in HER2 (IHC) and 6.5% in HER2 (FISH). Serial changes in genotype, from pre- and post-treatment biopsies, were also detected using NGS based Foundation Medicine platform, including acquired alterations in the ESR1 and PI3K pathway.

Conclusion: Serial changes in hormone receptor status and mutation profile are not uncommon among patients initially diagnosed with ER+/HER2 negative breast cancer, and some patients have been noted to have multiple changes over time. Further studies are needed to understand the mechanistic underpinnings governing the emergence of these alterations and their relationship to therapeutic resistance in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-06-04

Title: Molecular characterisation, subtype concordance and prognostic group assignment between patient-matched primary breast tumours and axillary lymph node metastases

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Body: Introduction

Currently the primary breast tumour is used for prognostic profiling and as a monitor of response to therapy but how often does the molecular profile of the primary cancer reflect the molecular profile of nodal metastases? No previous study has investigated in detail the genomic profile of matched primary breast cancer (P) and nodal metastases (N) and correlated these with outcome. The aim of this study was to investigate whether the mRNA profiles of matched P and N differ significantly.

Methods

RNA was extracted from core biopsies from primary breast tumours and paired metastatic axillary lymph node samples from both FFPE blocks and fresh frozen samples. RNA was labelled and hybridised to Illumina HT-12 BeadChips to create a dataset consisting of one primary and one or two matched nodal metastasis, totalling 68 samples from 31 patients. Data was processed and corrected for batch effects, then analysed using the statistical programming language R. Clinical data on progression free and overall survival was collected from electronic and medical case note review.

Results

Unsupervised hierarchical clustering of the 500 most variable genes in each sample grouped only 12 of 31 P&Ns (39%) together, meaning in the majority of patients their P or N more resembled a cancer from another patient than its own paired P or N.

The number of genes with greater than 2 fold change (>2FC) between P&N was used to categorise paired samples into 'least changed' (<130 genes with >2 FC) and 'most changed' (>370 genes with >2FC) groups. Multidimensional scaling of the 500 most variable genes in the most changed group (n=10) showed consistently that nodal metastases differed molecularly from the primary cancer.

When categorised by Sorlie centroid, 12 of 31 patients (39%) had a different molecular subtype in N compared with P. N tended to be a poorer prognostic subtype than P. 50% had luminal A primaries paired with luminal B nodes. The remaining 50% changed in other non-consistent patterns.

6 patients had 2 N samples to analyse alongside P. 4 of these (67%) had the same subtype in all 3 samples, and a further 1 the same 2Ns (luminal B) which differed from P (luminal A). The final had luminal A P paired with 1 luminal A and 1 luminal B Ns.

There was no evident correlation between the least changed and most changed groups and progression free and overall survival. This may however reflect the short term follow up.

Discordance between P and N in expression of ESR1 was 32%; PGR 19% and ERBB2 16%.

Conclusions

This study of gene expression change in matched primary breast cancers and synchronous metastatic paired axillary lymph nodes shows that molecular subtype differs in 39%. 50% of nodes had a poorer prognostic subtype than their primary. Expression of ESR, PGR and ERBB2 differs in up to 32%

Classifying cancer molecular phenotype and estimating prognosis based only on the primary cancer misclassifies significant numbers of patients. Classification of prognosis, and treatment based on the nodal metastasis may provide better information on which to base treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-06-05

Title: Breast cancer organoid cultures preserve intra-tumor heterogeneity and reveal intrinsically resistant phenotypes to standard chemotherapies

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Body: Breast cancer intra-tumor heterogeneity contributes to chemotherapy resistance and decreased patient survival, yet no reliable *in vitro* models exist to study this phenomenon. To address this need we developed an *in vitro* 3D organoid culture system using primary human breast cancer tissue. A major difficulty in the development of such models is to identify robust *in vitro* conditions that preserve the breast cancer phenotypes observed *in situ*. To address this challenge we used quantitative immunofluorescence imaging to compare the cellular phenotypes in the starting tumor tissue with those observed in the tumor organoids cultured in 3D. We utilized a clustering algorithm and utility function to quantitatively assess whether tumor organoids generated *in vitro* faithfully recapitulated intra- and inter-tumor heterogeneity of the tumor tissue *in situ*. This approach generated a normalized score that reflects tissue-organoid similarity. To test the sensitivity of our method to overall changes in tissue phenotype we focused on three distinct breast cancer subtypes distinguished by expression of estrogen receptor (ER), progesterone receptor (PR) and amplification of ERBB2/HER2 (HER2). Using our approach, we successfully recapitulated the tumor phenotypes present in ER+, ER+/HER2+ and triple negative breast cancer. We discovered that EGF preserves the TNBC phenotype, whereas AREG is required for recapitulating the phenotype of ER+ and ER+/HER2+ breast cancers. Additionally, our data demonstrate that HER1 ligands drive inter- and intra-tumor heterogeneity. To investigate how intra-tumor heterogeneity contributes to therapy responses we treated organoids with standard agents used clinically to treat each of the distinct subtypes. For all tumor subtypes we observed differential vulnerabilities between patients to drug treatments. Importantly, our analysis identified divergent cellular phenotypes that have various sensitivities to chemotherapies. Taken together, our methodology provides an unprecedented view of intra-tumor heterogeneity and allows for the investigation of chemo-resistance mechanisms. Further, this approach will provide a powerful tool, which will enhance the identification of novel therapies and facilitate personalized medicine.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-06-06

Title: Evidence for tumor heterogeneity and clonal evolution during invasive progression of breast cancer

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Body: Purpose: Intratumoral heterogeneity is well recognized to be an important driver of treatment resistance and metastasis. We undertook this N of one study to measure the degree of heterogeneity in a single large preinvasive lesion with an invasive component to determine the relationship between tumor heterogeneity, spatial distribution, clonal evolution, and invasive progression.

Methods: We identified a patient with extensive DCIS measuring 7.5 cm, associated with 1.5 cm of an invasive component. We segregated the tumor sample into 32 unique blocks with precise geospatial localization; invasive cancer was identified in 3 of 32 blocks. NGS libraries were made from FFPE derived DNA (20-40ng) for full exome sequencing and hybridization to a 4.8 million element SNP array. All data were analyzed and a phylogenetic tree was constructed.

Results: The sequence data was analyzed with Platypus[1] and 3922 somatic mutation sites were found in total. These sites were concatenated into one sequence for each sample. Then a Neighbor-Joining tree was built with a Jukes-Cantor model and 1000 bootstrap replicates using FastME 2.0[2], to assess the reliability of the tree (Figure 1). Phylogenetic analysis revealed that invasive cancer evolved twice, independently. Dense sampling allowed reconstruction of the temporal order of mutations that accumulated in the cell lineage of the invasive cancers. Furthermore, the phylogeny revealed that distant regions may be closely genetically related, the oldest parts of the tumor were in the interior of the tumor, and that the invasive tumors evolved near the oldest parts of the tumor, rather than at the expanding front.

Conclusions: Extensive sampling and sequencing of a single tumor yields important insights about tumor heterogeneity and tumor progression for DCIS to invasive cancer. Foci of invasion were geospatially associated with preinvasive regions of progressively higher mutational load.

1. Rimmer A, Phan H, Mathieson I, Iqbal Z, Twigg SR, Wilkie AO, McVean G, Lunter G, Consortium W: **Integrating mapping-, assembly-and haplotype-based approaches for calling variants in clinical sequencing applications.** *Nature genetics* 2014, **46**(8):912-918.

2. Lefort V, Desper R, Gascuel O: **FastME 2.0: a comprehensive, accurate, and fast distance-based phylogeny inference program.** *Molecular biology and evolution* 2015:msv150.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-06-07

Title: Clinical and molecular relevance of intra-tumor genetic heterogeneity in breast cancer: Integrative analysis of data from The Cancer Genome Atlas

Ma D, Jiang Y-Z, Liu X-Y, Liu Y-R, Yu K-D and Shao Z-M. Fudan University Shanghai Cancer Center, Shanghai, China; Cancer Institute, Fudan University Shanghai Cancer Center, Shanghai, China and Shanghai Medical College, Fudan University, Shanghai, China.

Body: Background: Intra-tumor heterogeneity (ITH) plays a pivotal role in driving breast cancer progression and therapeutic resistance. Emerging evidence has indicated that the extent of genetic heterogeneity may serve as a clinically useful biomarker. While several studies have suggested the prognostic value of ITH in several cancer types, the clinical significance of genetic ITH and molecular portraits that correlated with different ITH levels were poorly understood in breast cancer. The establishment of algorithms estimating genetic ITH based on sequencing of bulk tumor DNA offered us an opportunity to explore the clinical implication of ITH in large breast cancer cohorts and, for the first time, to use integrative genomic analyses to reveal molecular portraits related to intra-tumor genetic heterogeneity.

Methods: We assessed 916 female breast cancer patients from The Cancer Genome Atlas. Mutant-allele tumor heterogeneity (MATH) values were calculated from whole-exome sequencing data. We used integers nearest to the tertiles of the MATH values as cutoff points to divide the patients into three groups nearly equal in size. The association between MATH value and clinical characteristics was evaluated, followed by survival analyses in these different MATH groups. We then compared the rates of total non-silent somatic mutations among the different MATH groups, and further determined the mutations independently associated with high MATH by logistic regression adjusting for T classification and clinical subtypes. Similar methods, superadding somatic copy number alteration (SCNA) burden in logistic model, were used to evaluate SCNA events that were significantly associated with high MATH level. Gene enrichment between the high and rest MATH groups was analyzed using Gene Set Enrichment Analysis.

Results: The patients were divided into low (MATH value lower than 33), intermediate (MATH between 33 and 46) and high (MATH higher than 46) MATH groups. High T stage, African American race, and triple-negative or basal-like subtype were associated with a higher MATH level (all $P < 0.001$). In hormone receptor-positive and human epidermal growth factor receptor-negative patients, the high MATH group showed a tendency toward a worse overall survival ($P = 0.052$); however, while in triple-negative breast cancer, both high and low MATH indicated a worse outcome ($P = 0.032$). Furthermore, the TP53 mutation rate increased as MATH was elevated ($P < 0.001$), whereas CDH1 mutations were correlated with a lower level of MATH ($P = 0.002$). Several focal and arm-level SCNA events were more common in the high MATH group, including Chr8q24 with only the MYC gene in the "peak" region. Similarly, high MATH was associated with gene set enrichment related to the MYC pathway and proliferation.

Conclusion: Our study extended the knowledge concerning the clinical role of ITH in breast cancer, especially the distinct pattern of prognostic values in different clinical subtypes, which may help promote the clinical utilization of genetic ITH. Our attempt at exploring the molecular features related to ITH might provide clues for the source and consequences of ITH, inspiring subsequent experiments investigating the laws underlying tumor heterogeneity.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-06-08

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-06-09

Title: Relationships between breast cancer subtypes and expression of autophagy related genes

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Body: Background:

Breast cancer (BC) is a heterogeneous disease and can be classified according to the expression of four genes: *estrogen receptor (ER)*, *progesterone receptor (PGR)*, *human epidermal growth factor receptor 2 (HER2)* and marker of proliferation *KI67*. Four groups of BC have been described: Luminal A (ER^+ or PGR^+ , $KI67^-$ $HER2^-$), Luminal B (ER^+ or PGR^+ , $KI67^+$ or $HER2^+$), $HER2$ (ER^- $HER2^+$) and triple negative (ER^- PGR^- $HER2^-$). Autophagy is a lysosomal degradation pathway which plays a crucial dual role in tumorigenesis, producing pro-survival or pro-death activity. Altered autophagy has been observed in BC but no study has described transcript level variations of "autophagy genes" according to sub-groups. In order to further explore those alterations, we analyzed gene expression of 40 "autophagy genes" in normal and tumor cells using public transcriptomic data.

Samples and Methods:

5497 transcriptomes were obtained from raw data downloaded from public databases. Two distinct Affymetrix series were built after GC-RMA normalization: one from HG-U133A arrays (n=2806) and one from HG-U133 plus2 arrays (n=2691). Each series was standardized with the Aroma R package that was designed to normalize multicenter extremely large Affymetrix data sets. The same computations were performed in the two data set in order to cross-validate the results. Samples were classified with a reduced number of genes (such as for St Gallen classification) and with classifiers using 50 or more than 300 genes (PAM50, CIT and IntClust centroids).

Results:

In each of the two series, there were about 500 Basal, 300 $HER2$, 1800 Luminal tumors and 100 normal epithelial breast cells. Positivity or negativity of four genes (*ER*, *PGR*, *KI67*, *HER2*) produced robust classifications concordant with PAM50 classification based upon centroids. Within the 40 "autophagy genes" studied, at least 10 genes were significantly correlated with one or two sub-groups: *ATG3* and *ATG9A* were associated with ER^- tumors including basal group; *ATG2B*, *BECN1* and *ULK2* were positively correlated with ER expression and luminal subtypes; *ULK1* and *RAB24* were associated with ER^+ $HER2^+$ tumors and *MAP1LC3B* and *ATG5* with ER^-HER2^+ subgroup; *GABARAPL1* was more expressed in normal breast tissue than in cancer cells.

Discussion:

Exploring autophagy in large-scale transcriptome data, we confirmed a previous result showing that *GABARAPL1* expression is reduced in breast cancer cells compared with the normal epithelial cells. Interestingly, transcript levels of "autophagy genes" were not evenly distributed among the different tumor subtypes. For instance, some genes were preferentially expressed in ER^+ subtypes and others in ER^- tumors, suggesting that autophagy might play different roles in the different subgroups, providing different potential targets for therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-06-10

Title: Next generation sequencing (NGS) reveals high mutation rates in all established breast cancer subtypes with subtype-specific patterns

Bermanian V, Sauer T, Joel T, Katja V, Vessela K, Ida B and Jürgen G. Gene Technology; Pathology; Breast and Endocrine Surgery; EPIGEN; Oncology; Akershus University Hospital, Lørenskog, Norway and University of Oslo, Institute of Clinical Medicine, Campus AHUS, Oslo, Norway.

Body: Introduction. Next-generation sequencing (NGS) technologies offer the possibility for assessment of multiple genes for their somatic mutations and may elucidate the driver genetic variations involved in carcinogenesis and disease progression. Among the various subtypes of breast cancer (BC), the triple-negative subgroup (TNBC) is characterized by poor prognosis and a lack of reliable tumor markers when compared to the luminal-A/-B and HER-2 positive subtypes. The aim of this study was to characterize genetic variations in primary BC obtained from a cohort of 159 Norwegian patients using a NGS panel consisting of 44 BC relevant genes. Our goal was to compare the genetic variations between BC subtypes in general with special emphasis on the TNBC subtype (over 40% of the patients). **Methods.** Genomic DNA was extracted from paraffin embedded formalin fixed (FFPE) tissue obtained from 160 consecutive patients diagnosed with a primary BC at our hospital. The DNA samples were analyzed by next-generation sequencing (NGS) using Human Breast Cancer GeneRead DNAseq Targeted Panel V2 (Qiagen). The panel consists of a collection of PCR primers for targeted enrichment of the coding region of 44 genes commonly mutated in BC. Target enrichment and library construction was performed according to the GeneReader workflow (Qiagen) and paired end sequencing was performed on a NextSeq 500 sequencer (Illumina) running 2 x 150 bp chemistry Version 2. Data analysis including alignment to the reference genome hg19 and variant calling was performed using Qiagen's online Ingenuity Variant analysis. **Results.** The ingenuity variant analysis classified the genetic mutations according to their clinical significance into four groups: pathogenic, likely pathogenic, benign and likely benign. We present only mutations in genes that are characterized as pathogenic or likely pathogenic, where the term "likely pathogenic" indicates greater than 90% certainty of the mutation being pathogenic (as defined by the American College of Medical Genetics and Genomics). Genetic variations were mostly observed in a subset of 44 genes included in the breast cancer panel. The tumor suppressor genes TP53 as well as BRCA1 and BRCA2 represented the highest mutation rates (>5%) among all BC samples. Interestingly, additional genes potentially playing a pivotal role in BC biology like EP300 were also found to be mutated at a high rate in TNBC. The biological significance of the EP300 gene remains unknown. Additionally, comparing the mutation rates of several genes like TP53, PIK3CA, BRCA2, ATM, RET and EGFR between established BC subtypes showed significant differences. **Conclusion.** Next generation sequencing of samples obtained from primary breast cancer tumors confirmed a high level of pathogenic or likely pathogenic mutations in a subtype-specific pattern involving genes like TP53, BRCA1/2, ATM, EGFR, RB1 and PIK3CA.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-06-11

Title: A targetable EGFR-driven tumor-initiating program in breast cancer

Savage P, Saleh SMI MI, Wang Y-C, Revil T, Badescu D, Liu L, Iacucci E, Zuo D, Bertos N, Munoz-Ramos V, Asselah J, Meterissian S, Omeroglu A, Hébert S, Kleinman C, Park M and Ragoussis J. Goodman Cancer Research Centre, McGill University, Montreal, QC, Canada; Genome Québec Innovation Centre, McGill University, Montreal, QC, Canada; McGill University Health Centre, Montreal, QC, Canada and Lady Davis Institute, Jewish General Hospital, Montreal, QC, Canada.

Body: Background: Inter- and intra-tumour heterogeneity underlies variability in therapeutic response. Although targeting of the epidermal growth factor receptor (EGFR) in breast cancer has failed to demonstrate clinical efficacy at the population level, complete and durable responses have been reported at low frequencies. The molecular determinants of these responses are unknown, but are of importance in the era of precision medicine. **Results:** We performed a patient-derived xenograft (PDX) clinical trial with gefitinib in a breast cancer PDX cohort. Consistent with clinical trial data, gefitinib exhibited limited efficacy across most models. One PDX, however, demonstrated a complete and durable (>6 months) clinical response, and was subject to deep molecular profiling to identify determinants of response. Exome sequencing revealed no single nucleotide variants or copy number alterations in EGFR pathway members. EGFR was differentially expressed between the two major cellular subpopulations identified by single-cell RNAseq and this cellular heterogeneity in EGFR expression was validated immunohistochemically. Fluorescence-activated cell sorting of the EGFR^{hi} subpopulation revealed cells with enhanced stem-like properties, including ALDH activity, sphere-forming capacity *in vitro*, ability to form tumours *in vivo* and seeding lung micrometastases from orthotopically transplanted tumours. Tumourspheres derived from EGFR^{hi} cells developed into mixed EGFR^{hi} and EGFR^{lo} subpopulations, as did macrometastases, supporting that EGFR^{hi} subpopulation can self-renew and re-populate. Analysis of expressed SNVs in the single-cell RNAseq data, filtered by variants identified from exome sequencing, showed no clonal segregation, supporting a non-clonal origin of the functionally distinct EGFR^{hi} and EGFR^{lo} subpopulations. This EGFR-driven tumour initiating cell program was observed in independent PDX models, some which showed growth inhibition in response to gefitinib. **Conclusions:** Using bulk and single-cell genomic profiling, we identified and functionally validated an EGFR-driven tumour-initiating program in a subset of aggressive breast tumours, which may be predictive of gefitinib sensitivity. This contradicts traditional beliefs that good therapeutic targets are homogeneously expressed, in that we show that a target displaying intra-tumour heterogeneity can be effective so long that it is expressed in the tumour-initiating population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-01

Title: A pan-cancer perspective of functional proteomics provides novel information content for uncommon breast cancer subtypes

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Body: Cancer cell lines (CCLs) serve as models to study the functional consequences of the genomic lesions in patients and as screening platforms for prediction of drug response. While genomic and transcriptomic data have proven to be useful predictors, the ability of these omics platforms to predict protein level and function is limited. Furthermore, since proteins are the targets of the majority of the targeted therapies, protein levels and importantly protein function would be expected to provide more powerful predictions than DNA or RNA data. While large scale genomic and transcriptomic data linked to drug sensitivity are available for over a thousand CCLs, proteomic data is available for only a small subset of lines. Here we performed proteomic profiling of 736 cell lines using reverse-phase protein arrays (RPPAs) with approximately 300 antibodies providing an unbiased sparse representation of the majority of signaling pathways.

The functional proteomic analysis revealed 10 protein-based clusters across all cell lines. Similar to human tumors, the breast cell lines fell into three major clusters representing basal-like, luminal/Her2-amplified and claudin-low breast cancer subtypes. The basal-like and claudin-low clusters contained all of the representative breast cancer cell lines as well as a much larger number of other CCLs. For example, the 6 claudin-low breast cancers analyzed reside in an EMT cluster, in which only 8/126 are breast cell lines. However, the complete cluster including multiple non-breast cancer cell lines recapitulated mRNA and protein features of claudin-low breast tumors, including a high EMT signature and low level of hormone receptor pathway activity. We thus explored whether we could gain power for linking the limited number of basal and claudin-low breast cancer cell lines to therapeutic sensitivity by assessing patterns of drug sensitivity in each cluster for both the breast and non-breast cancer cell lines in the cluster. We explored drug sensitivity of 481 therapeutic compounds from the Cancer Therapeutic Response Portal (CTRP v2) and demonstrated that the non-breast cancer and breast cancer cell lines in each cluster provided similar patterns of drug sensitivity. For example, Claudin-low/EMT cell lines of both breast cancer and non-breast cancer origin showed decreased sensitivity to PI3K/mTOR inhibitors compared to luminal breast cancers ($p < 0.05$ for 4 mTOR inhibitors) and drugs targeting EGFR family compared to basal cell lines ($p < 0.05$ for 7 EGFR/ERBB2 inhibitors). Thus it is possible to gain information by characterizing cell lines with similar patterns of protein expression and provide important information related to drug sensitivity of uncommon breast cancer lineages. The functional proteomic analysis provides a wealth of information that complements the genomic and transcriptomic studies of cancer cell lines, and demonstrates the opportunity to leverage cell line 'pan-cancer' proteomic patterns to improve characterization of specific breast cancer subtypes. To facilitate broad access to these data, we developed a user-friendly data portal, the MD Anderson Cell Lines Project (MCLP), that provides both data analysis and download (<http://ibl.mdanderson.org/mclp/>).

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-02

Title: Chemotherapy (CT) decision in patients (pts) with node-positive (N+), ER+, early breast cancer (EBC) in the wake of new ASCO guideline – A different take on the evidence for the 21-gene recurrence score (RS) assay

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Body: Background: The use of molecular tools for prognosis and prediction of CT benefit in EBC has increased the complexity of decision making. The 21-gene RS (Oncotype DX) is included in the ASCO (2007) and NCCN guidelines (2006) for prognosis (risk of distant recurrence [DR]) and prediction of CT benefit in N0, ER+ EBC. In 2015, the NCCN added that the RS assay could be considered for select patients with 1-3 N+, ER+ EBC. Recently the ASCO BC biomarker/guideline group (J Clin Oncol 2016) advised that the “clinician should not use the 21-gene RS to guide decisions” and called the evidence quality “intermediate” and the recommendation “moderate” based on review of 2 N+ studies. It also advised no change in N+ clinical practice until the prospective SWOG S1007 study (RxPONDER) matures in several years. These discordant recommendations have led to major confusion among physicians, pts and payers. To address this controversy we herein report a comprehensive analysis of the body of evidence regarding the clinical utility of the RS in N+, ER+ EBC.

Methods: All published studies involving N+, ER+ EBC with RS data were analyzed by type of study design and category of trial (validation, supportive, decision impact, cost-effectiveness, and prospective outcomes).

Results: 30 studies provided clinical evidence supporting the value and utility of the RS in N+, ER+ pts. 7 studies employed a prospective-retrospective design or were prospective outcomes with clinical utility in >8000 N+ pts (Table). 23 additional studies assessed the impact of RS on CT decisions or cost-effectiveness.

Study in N+/ER+	Type of study	N	Endpoints/results
SWOG S8814 (Lancet Oncol 2010)	Pro-retro	367	10-year DFS and BCSS: RS predicts risk of DFS event, BC death, and CT benefit (none to slightly worse if very low risk RS and 1-3 N+)
TransATAC (JCO 2010)	Pro-retro	306	9-year DR: RS predicts risk of DR in pts treated with ET without CT
ECOG E2197 (JCO 2008)	Pro-retro	232	5-year DR: RS predicts DR risk in CT+ET treated pts
NSABP B-28 (ASCO-BCS 2012)	Pro-retro	1065	10-year DRFI: RS predicts DR risk in CT+ET treated pts
PACS-01 (ASCO 2014)	Pro-retro	530	5-year DRFI/DFS: RS predicts DR risk in CT+ET treated pts
SEER (npj BC 2016)	Prospective outcomes	4691	5-year BCSM: RS predicts BCSM; pts with RS <18 (Nmi, 1-3 N+) had 1.0% BCSM
WSG PlanB (JCO 2016)	Prospective outcomes	1198 (1088 N1-3/110 N0)	3-year DFS: 98.4% in high risk N0/N+ ER+, RS <12 group and 97.5% in RS 12-25 group (5-year DFS 94.0% in both RS groups)

BCSM: BC-specific mortality; BCSS: BC-specific survival; DFS: disease free survival; DR: distant recurrence; DRFI: distant recurrence free interval; ET: endocrine therapy; Nmi: micrometastases; pro-retro: prospective-retrospective.

Conclusions: The 21-gene RS has now been studied in >10,000 N+, ER+ EBC pts across 30 studies worldwide, including 2

prospective outcomes studies in >5000 pts, confirming that the RS consistently identifies low risk 1-3 N+ pts in whom CT can effectively and safely be avoided. This evidence suggests that ER+ pts with few N+ and low RS should have a discussion of the pros and cons of adjuvant CT until the results of RxPONDER provide a definitive answer in several years.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-03

Title: Mesenchymal subtype negatively associates with the presence of immune infiltrates within a triple negative breast cancer classifier

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Body: Introduction: Lehmann and colleagues (Lehmann et al., 2011) devised a gene expression classification system for triple negative breast cancer (TNBC) consisting of seven subtypes—IM, BL1, BL2, LAR, M, MSL, and UNS (unselected). We (Ring et al., 2016) recently modified this original algorithm of 2188 gene subtyping into a 101-gene algorithm. In addition to a reduction of genes, the 101-gene algorithm has two methodological differences: first, the immunomodulatory (IM) signature was treated not as a subtype but rather as a binary feature of one of the other subtypes (e.g. BL1/IM+, LAR/IM-); second, when tumors—by a predefined correlation coefficient—showed traits of more than one subtype, both subtypes were reported as “dual subtypes.”

Aim: Our aim was to apply the 101-gene algorithm for TNBC subtyping and to establish the relation of TNBC subtypes with their IM-status across several independent data sets.

Methods: 951 patients from four independent TNBC cohorts with available gene expression data were analyzed by the 101-gene algorithm. Of these 848 were classified with at least one subtype.

Results: The distribution of the 5 TNBC subtypes in both single and dual subtypes was 47%,10%,15%,18%,11%, for BL1, BL2, LAR, M, and MSL respectively. The majority of cases gave only one subtype (572, 67%) with M (Mesenchymal) being 9% (n=54) of these. Given this frequency of 9% of M as a baseline, in the remaining 276 (33%) cases with dual subtypes, the expectation that M would be one of the two is 11% (64 subtype calls). However, M is one of the two of the dual subtypes at a much higher frequency of 40% (222 subtype calls, Chi-Squared, $P < 0.0001$). IM+ is a common feature across these cohorts (n=310 or 37%). When examining the IM feature within the patients exhibiting the M subtype as either a single subtype or one of the two dual subtypes (n=276, 33%), IM positive tumors are never of the M phenotype (Chi-Squared, $p < 0.0001$).

Conclusions: We further have confirmed with the 101-gene algorithm that the IM signature inversely associates with the M subtype as it has been observed with the 2188 gene algorithm (Lehmann et al., 2016). Moreover, the M signature is occasionally a confounder of other subtypes however still identifies those tumors negative for immune infiltrates. This raises important opportunities to understand the relationships between intrinsic tumor biology reflected in TNBC subtypes and their interaction with variable immune cell stroma which are the subject of ongoing analyses.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-04

Title: Unique overlapping subtypes of triple-negative breast and ovarian cancers and sensitivity of “mesenchymal-like” cancers to HSP90 inhibition is revealed by integrated gene expression and drug sensitivity profiling

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Body: Next-generation sequencing and gene expression signature analysis of primary tumors from The Cancer Genome Atlas (TCGA) revealed striking similarities between triple-negative breast cancer (TNBC) and ovarian cancer (OVCA). We hypothesized that these similarities may reveal transcriptionally-identifiable subgroups within a mix of TNBC and OVCA that are uniquely sensitive to certain drugs.

To test this hypothesis, gene expression profiles for TNBC and OVCA cell lines in the Cancer Cell Line Encyclopedia were analyzed by unsupervised hierarchical clustering, which revealed two major unique subgroups containing both TNBC and OVCA cell lines that clustered according to the previously defined Mesenchymal and Basal subclasses of TNBC. Differential gene expression between “Mesenchymal-like” and “Basal-like” TNBC/OVCA cell lines was used to generate a gene signature that was subsequently validated using gene expression data in the Genomics of Drug Sensitivity in Cancer (GDSC) database. Drug sensitivity data from GDSC was then utilized to profile differential sensitivity of “Mesenchymal-like” and “Basal-like” cell lines to the 99 anti-cancer drugs with coverage of >50 breast and ovarian cell lines. Mesenchymal-like TNBC/OVCA cells were uniquely sensitive to HSP90 inhibition compared to Basal-like TNBC/OVCA cells for both HSP90 inhibitors in GDSC: CCT018159 ($p < 0.001$) and 17-AAG ($p = 0.012$). Strikingly, Mesenchymal-like TNBC/OVCA cells were most sensitive to HSP90 inhibition among all 33 solid tumor cancer lineages in GDSC, as well as other subgroups of breast and ovarian cancers.

Differential sensitivity of Mesenchymal-like and Basal-like TNBC/OVCA cells to HSP90 inhibition with 4 agents (CCT018159, 17-AAG, AT13387, PU-H71) was validated using growth assays for 12 cell lines (6 of each subgroup) previously characterized in GDSC. To further validate the predictive value of our gene signature, gene expression data for 6 TNBC/OVCA cell lines with uncharacterized drug sensitivity was used to classify cell lines as “Mesenchymal-like” ($n=4$) or “Basal-like” ($n=2$). Our gene signature successfully predicted differential sensitivity to HSP90 inhibition, with the 4 Mesenchymal-like cell lines displaying greater sensitivity to HSP90 inhibition. The gene signature has been used to select PDX models with predicted differential sensitivity to HSP90 inhibition, and treatment studies are ongoing.

In summary, we developed a novel approach to generate a gene expression signature that robustly predicts sensitivity to HSP90 inhibitors in preclinical models of TNBC and OVCA. HSP90 thus represents a therapeutic opportunity in an overlapping subpopulation of TNBC and OVCA. Our approach to identify novel combinations of drugs and histology/lineage-independent cancer subgroups may be used to discover new therapeutic opportunities in other cancer types.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-05

Title: Integrated transcriptional analysis of the triple negative 'proliferation paradox': High proliferation, chemosensitivity, and poor prognosis

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Body: Background: In triple-negative breast cancers (TNBC), high proliferation is associated with greater chemosensitivity but, paradoxically, also associated with poor prognosis. We hypothesized that this subset of TNBC has distinct transcriptional features that contribute to poor prognosis.

Approach: To evaluate transcriptional signatures associated with this 'proliferation paradox,' we identified 17 study cohorts of TNBC treated with neoadjuvant chemotherapy (NAC) that reported receptor status, pathologic response, and had expression data from biopsies obtained prior to NAC (n=446). In 6 studies, distant metastasis-free survival (DMFS) data was available for 235 patients with a median follow-up of 31.2 months. We calculated scores for 135 published gene expression signatures for each tumor and evaluated the association with response to chemotherapy and DMFS.

Results: Using recursive partitioning to develop a model of response using a training set (n=340), six of the 135 expression signatures stratify primary tumors into four groups based on signatures of proliferation, BRCA1 mutation, immune, luminal, Ras, and PI3K phenotypes (Table 1.). Response to NAC ranged from 11% to 61% pCR/RCB-I and results were highly concordant when applied to a validation set (n = 106, p = 0.006). The group that was highly proliferative but chemoresistant ('resistant' group) had a distinct transcriptional profile, including lower 'BRCA-ness' and DNA damage expression signatures with higher Ras and stem cell signatures. The 'resistant' group had the poorest DMFS (HR 2.48 [1.52-4.06]; log-rank p=0.002) and this poor survival was validated among chemotherapy-treated TNBCs in a separate dataset, METABRIC. Analyses of only patients with residual disease after NAC demonstrated that the 'resistant' group remained poorest prognosis, with median DMFS of only 31 months from diagnosis.

Conclusions: Using a novel approach to categorize primary TNBC tumors based on six signatures, we can effectively distinguish subgroups with higher versus lower pCR rates. One specific group demonstrated high proliferation but low response to chemotherapy and particularly poor survival. This group demonstrates expression signatures implicating DNA damage repair, stemness, and Ras pathway activity as potential mediators of the phenotype. We identify specific molecular characteristics for investigation in patients within a poor prognosis subgroup of TNBC.

Table 1. Proportion Pathologic Complete Response or RCB-I and Survival

	Low Prolif	High Prolif / Resistant	High Prolif / Sensitive	High Immune
Signature Stratification	Low GGI + High Luminal	High GGI + Low BRCA1mut or High Ras	High GGI + High PI3K or Low Ras	High TNBC Immune
pCR/RCB-I rate: Training Set	11/105 (10.5%)	26/127 (20.5%)	42/81 (51.9%)	16/27 (59.3%)
pCR/RCB-I rate: Validation Set	3/23 (13.0%)	11/45 (24.4%)	13/29 (44.8%)	6/9 (66.7%)
pCR/RCB-I rate: TOTAL	14/128 (10.9%)	37/172 (21.5%)	55/110 (50.0%)	22/36 (61.1%)
Overall Survival (n=235)				
Hazard Ratio (95% CI)	1.62 (0.99-2.64)	2.48 (1.52-4.06)	(ref.)	0.47 (0.29-0.77)

Signatures GGI (Sotiriou, JNCI 2006); Luminal (Lim, Nat Med 2009); BRCA1 mutation (van't Veer, Nature 2002); Ras (Pratilas, PNAS 2009); PI3K (Gatza, PNAS 2010), TNBC Immune (Lehmann, JCI 2011)

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-06

Title: Immune signatures define and affect prognosis in triple-negative breast cancer subtypes

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Body: Background: The tumor immune microenvironment is formed by many distinct and interacting cell populations, and its composition may predict patient's prognosis and response to therapies. Triple negative breast cancer (TNBC) is a heterogeneous disease in which different genomic subgroups have been described (Lehmann, *et al. J Clin Invest.2011*). Our aim was to integrate the composition of the tumor immune microenvironment with the molecular TNBC subtypes.

Methods: We retrospectively analyzed the composition and the functional orientation of the immune microenvironment of 963 TNBC tumors clustered in 4 main TNBC subgroups (Basal-Like [BL1/2], Immunomodulatory [IM], Mesenchymal/Mesenchymal Stem-Like [MS], Androgen Receptor [AR]) from independent cohorts using transcriptomic profiling. TNBC were stratified based on 9 different immune signatures (Natural Killer [NK], Dendritic Cells [DC], T-Cells [TC], B-Cells [BC], T-Cytotoxic [TC], Interferon [IF], Nuclear Factor-kB [NF-kB], anti-tumor Macrophages [M1] and pro-tumor Macrophages [M2]. We validated our findings using immunohistochemistry.

Results: We report that TNBC molecular subgroups and specific microenvironmental immune signatures are highly correlated. The IM was enriched of almost all the immune modules. On overall TNBC population, the TC ($P = 0.01$), IF ($P = 0.007$) and M1 ($P = 0.001$) signatures were associated with good prognosis. The BL subgroup with a good prognosis was characterized by overexpression of genes specific to M1-macrophages ($P = 0.004$) and TC-lymphocytes ($P = 0.01$). In contrast, the poor-prognosis MS expresses markers of cells of monocytic origin and significantly associated with the M2 signature ($P = 0.01$). The MS subgroup also displays an inflammatory and immunosuppressive signature. Pathological examination revealed that the MS subtype is characterized by a high density of pro-tumor macrophages that likely produce chemokines and cytokines which favor tumor-associated inflammation, resulting in a poor prognosis. In contrast, the AR exhibits low immune and inflammatory signatures.

Conclusions: The distinct immune orientations of the TNBC molecular subtypes pave the way for tailored immunotherapies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-07

Title: Gene expression signatures and immunohistochemical subtypes add prognostic value to each other

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Body: Background: We have previously demonstrated that gene expression signatures and Ki67 stratify the same breast tumour into opposing good/poor prognosis groups in approximately 20% of cases. Given this, we hypothesized that the combination of a clinically relevant gene signature and IHC markers may provide more prognostic information than either classifier alone. We tested this hypothesis in a large independent cohort of Swedish breast cancer patients with long-term follow-up data.

Methods: We assessed Ki67, ER, PR, HER2 and the research versions of the Genomic Grade Index (GGI), Mammaprint, cell-cycle score (CCS), Recurrence Score (RS) and PAM50 gene expression classifiers on matching TMA and microarray data in a Swedish breast cancer cohort of 623 patients. Change in likelihood-ratio ($\Delta LR\chi^2$) was used to first determine the additional prognostic information provided by gene expression signatures beyond that provided by 1) Ki67 alone and 2) Ki67 plus ER, PR and HER2, grouped to form the IHC molecular subtypes. Secondly and conversely, we then determined the additional prognostic information provided by Ki67/IHC subtypes beyond gene expression signatures.

Results: Representative images from Ki67/gene signature contrast groups show tumours with high levels of Ki67 expression that are classified as good prognosis by gene signatures and conversely, tumours with low Ki67 that are classified into poor prognosis groups by gene signatures. In all patients (n=623), the majority of signatures provided statistically significant information beyond that of Ki67 alone, however only RS and PAM50 remained significant in the presence of the IHC subtypes ($\Delta LR\chi^2$ RS= 11.7 and PAM50 = 15.4; $P = 0.002$ and 0.004 , respectively). Conversely, IHC subtypes added prognostic information beyond gene signatures whilst Ki67 alone did not, a notable exception to this was PAM50.

Conclusions: In general, a combination of the IHC subtypes with gene signatures provides more prognostic information than either classifier alone when considering all breast cancer patients. Subsequent analyses will focus on patient subgroups including ER positive, node positive and ER positive, node negative groups, along with validation of our work in a second dataset of 253 patients.

Change in likelihood ratio with the addition of gene expression signatures to Ki67/IHC subgroups and vice-versa

	All Patients			All Patients	
Sig. added to Ki67:	Sig. $\Delta LR\chi^2$	P-value	Sig. added to IHC subtypes	Sig. $\Delta LR\chi^2$	P-value
GGI	6.0	0.014	GGI	2.5	0.108
Mammaprint	6.3	0.011	Mammaprint	1.1	0.279
RS	20.8	< 0.001	RS	11.7	0.002
CCS	1.7	0.409	CCS	2.0	0.360
PAM50	25.0	< 0.001	PAM50	15.4	0.004
Ki67 added to sig.:	Ki67 $\Delta LR\chi^2$	P-value	IHC added to sig.:	IHC $\Delta LR\chi^2$	P-value
GGI	1.6	0.205	GGI	14.9	0.001
Mammaprint	1.6	0.199	Mammaprint	15.3	0.001
RS	0.5	0.477	RS	12.6	0.005
CCS	4.1	0.041	CCS	16.1	0.001
PAM50	2.3	0.13	PAM50	6.1	0.107

Sig.: Gene expression signature; GGI: Genomic grade index; RS: Recurrence score; CCS: Cell cycle score.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-08

Title: Mixed ductal-lobular carcinomas of the breast: Abrogated cell adhesion in the clonal evolution from ductal to lobular morphology

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Body: Mixed ductal-lobular carcinomas (MDL) display both ductal and lobular morphology, and are a clear example of intratumour morphological heterogeneity. The evolution of MDL carcinomas is not well understood. There is a paucity of data surrounding the genetic origin of the different morphological compartments and it remains to be seen whether the coincident presentation of these distinct morphological entities represents two independent tumours that have collided (so called 'collision tumours'), or whether they arise from a common clone. We propose that clonal progression during the evolution of these tumours is associated with a change in phenotype. To address this, a cohort of 82 MDLs was studied for clinical, morphological and molecular features. Key findings include: i) MDLs more frequently co-exist with ductal carcinoma *in situ* (DCIS) than lobular carcinoma *in situ* (LCIS); ii) the E-cadherin-catenin complex was recurrently normal in the ductal component but aberrantly localised in the lobular component of the same tumour; iii) E-cadherin deregulation in the lobular component was almost always aberrantly located to the cytoplasm, conversely classic ILCs are typically completely negative for this molecule; iv) epithelial to mesenchymal transition marker expression was not associated with E-cadherin deregulation. Comparative Genomic Hybridisation (CGH) and exome sequencing was performed to investigate clonal relationships between the different intratumour morphologies and identify mechanisms underlying the change in phenotype. Our analysis revealed that i) all morphological components within a case are clonally related; ii) divergence of the morphological components may occur early during tumour evolution (where both DCIS and LCIS are present) or later during tumour progression (cases with only DCIS detectible); and iii) mutations were identified in genes such as *CDH1* and *ESR1*, and other breast cancer driver genes. Together, these data strongly support the concept that the disparate morphological components of these mixed tumours are clonally related, and are not the result of a collision event. Furthermore, we show that lobular morphology can arise via a 'ductal' pathway of tumour progression. The mechanisms driving the change in phenotype are yet to be fully elucidated, but there is significant intertumour heterogeneity and each case may utilise a unique molecular mechanism.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-09

Title: A multi-OMIC analysis to explore the impact of “actionable” genomic alterations on protein pathway activation: Clinical implication for precision medicine in metastatic breast cancer

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Body: Background: While genomic alterations are central players in tumor progression, proteins are the targets for precision therapy. The degree by which “actionable” genomic alterations translate into activated/altered proteins and pathway is still under investigation. Using a multi-OMIC approach from the SideOut 2 metastatic breast cancer (MBC) trial, this study explored the concordance between selected “actionable” genomic alterations and protein expression/activation.

Methods: Snap frozen biopsies from 29 MBC patients enrolled in a prospective phase II trial were used for this analysis. Exome WES and RNASeq data was processed using an in-house developed pipeline and identified amplification of CCND1 (6/29), FGFR1 (4/29), and FGF 3, 4, 5, and 19 (4/29) as some of most frequent “actionable” genomic alterations in our MBC cohort. Signaling analysis of the 29 cases was performed using Reverse Phase Protein Microarray coupled with Laser Capture Microdissection. Protein expression/phosphorylation was measured in a continuous scale and classified based on quartile distribution. Concordance between CCND1 amplification and Cyclin D1 expression, along with the activation of FOXM1 T600 and Rb S780, was explored. Amplification of the FGFR1 locus or its ligands was correlated with the level of activation/phosphorylation of FGFR1 Y653/654.

Results: While Cyclin D1 protein expression was greater than the population mean for 4/6 (67%) patients with CCND1 amplification, only 2/6 (33%) patients with CCND1 amplification had Cyclin D1 level within the top quartile of the population (n=29). FOXM1 T600 activation was independent from CCND1 amplification, with high levels of FOXM1 T600 predominantly in the CCND1 wild-type population. Only 1/6 (17%) patients with CCND1 amplification had FOXM1 T600 level similar to the top quartile of the population while a second patient was above the population median. Activation of Rb S780 was above the population median, but below the top quartile, in 2/6 (33%) CCND1 amplified patients. Similarly, none of the patients with activation of FGFR Y653/654 equal to the top quartile harbored an FGFR1 amplification. Only 1/4 (25%) patients carrying an FGFR1 amplification had an activation of FGFR Y653/654 above the population median. Similarly, 1/4 (25%) patients with FGF ligand amplification showed FGFR Y653/654 level within the top quartile while three patients had FGFR Y653/654 activation below the population median. No significant results were found between proteomic (below/above the median) and genomic characteristics by Fisher test ($p>0.05$).

Conclusion: Molecular genotyping of “actionable” cancer targets alone may be insufficient in predicting whether the actual drug target protein is expressed and/or activated in any given patient's tumor. Although these results need further validation, the combination of genomic and proteomic data may represent a more informative approach for identifying real molecular drivers of individual lesions as well as “actionable” protein/phosphoprotein targets in the absence of genomic events. Multi-OMIC approaches may lead to more effective stratification in precision medicine trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-10

Title: Prediction of 10yr distant recurrence (DR) using the Prosigna® (PAM50) assay in histological subgroups of a Danish breast cancer group (DBCG) cohort of postmenopausal Danish women with hormone receptor-positive (HR+) early breast cancer (EBC) allocated to 5yr of endocrine therapy (ET) alone

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Body: Background: The Prosigna (PAM50) risk of recurrence (ROR) score predicts 10yr DR in early breast cancer patients treated with ET alone (level 1 evidence). Invasive lobular breast cancer (ILBC) accounts for 10-15% of all breast cancer histological subtypes. Sporadic ILBC is characterized by somatic *CDH1* mutations with loss of E-cadherin and a majority of low proliferative ER positive/HER2 negative tumors consistent with Luminal A subtype. Here we examine the ability of PAM50/ROR to predict 10yr DR in postmenopausal women who following a diagnosis of HR+ early ILBC were allocated to 5yr of ET alone.

Methods: Using the population based clinical DBCG database FFPE primary tumor blocks, treatment, and follow-up data were collected from all patients diagnosed from 2000-2003 with HR+, postmenopausal EBC (N0-N1) who by nationwide guidelines were allocated to 5yr of ET alone, N=2,722 (1256 N0, 1466 N1). PAM50 intrinsic subtype classification (Luminal A, Luminal B, HER2-enriched, Basal-like) was conducted using the NanoString nCounter® Analysis System. Univariate and multivariate analyses tested the ability of PAM50 to predict DR. Patients were categorized as Low or High Risk based upon pre-specified ROR cutoff value of 40. HER2 positive tumors by immunohistochemistry were excluded from analysis.

Results: Median follow-up was 9.25 years. Risk of 10yr DR by ROR and PAM50 (Luminal A and Luminal B) is shown in the table by ILBC as compared to invasive ductal breast cancer (IDBC) type.

Cumulative incidence for 10 yr distant recurrence (DR)

Histological subtype	Risk Group % [95% CI] (N row%)		Intrinsic Subtype % [95% CI] (N row%)		Histological subtype % [95% CI] (N)
	Low	High	Luminal A	Luminal B	
Ductal	3.8 [2.5-5.6] (738 35%)	16.6 [14.4-18.8] (1388 65%)	6.6 [5.1-8.4] (1174 59%)	18.1 [15.2-21.3] (832 41%)	12.1% [10.6-13.7] (2126)
Lobular	9.7 [5.5-15.4] (181 53%)	23.9 [16.8-31.6] (159 47%)	12.7 [8.6-17.8] (256 77%)	24.1 [14.5-35.1] (77 23%)	16.4 [12.2-21.1] (340)

In this specific cohort the ILBC subgroup had a worse 10yr DR of 16.4% [12.2-21.1] as compared to IDBC of 12.1% [10.6-13.7] (Gray's test, $p = 0.046$). A significant difference regarding the distribution of ILBC into both High and Low Risk Group and intrinsic subtypes as compared to IDBC was identified ($p < 0.0001$). Molecular intrinsic subtype analysis for ILBC by Prosigna (PAM50) showed DR 12.7 [8.6-17.8] for Luminal A vs 24.1 [14.5-35.1] for Luminal B. The difference between ILBC subtypes was not significant ($p = 0.09$). Adding ROR to a Fine and Gray's proportional sub-hazards model containing clinical and pathological variables significantly improved the model for ILBC, (likelihood ratio: $p = 0.0001$); HR for a 20-point change in ROR = 1.84 [1.37-2.48] notable with DR 9.7 [5.5-15.4] for the Low Risk Group.

Conclusions: Following 5-yr of endocrine treatment alone patients with ILBC in this large population-based study had an inferior DR as compared to patients with IDBC and the apparent difference between ILBC and IDBC must be interpreted with caution.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-11

Title: Tumor genomic profiling of triple negative breast cancer during neoadjuvant chemotherapy: Results from a prospective trial of carboplatin and docetaxel

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Body: Background-The clonal evolution and effect of neoadjuvant chemotherapy on the mutational landscape of triple negative breast cancer (TNBC) is unknown. Inability to eradicate TNBC may be due to clonal progression and selection of cells fundamentally resistant to chemotherapy. In this study, we sought to decipher the genomic architecture of TNBC serially during neoadjuvant chemotherapy to distinguish pre- versus post-chemotherapy genotypes.

Methods-Tumor specimens were obtained from patients with stages II and III TNBC enrolled on an ongoing prospective neoadjuvant co-clinical trial (NCT02124902). Patients have a research biopsy at baseline, cycle 1 day 3 (optional), and at definitive surgery for those with residual disease. Patients are treated with docetaxel 75mg/m² and carboplatin AUC6 cycled every 3 weeks X six cycles. Definitive surgery is 3-5 weeks after chemotherapy. The primary endpoint is pathologic complete response rate. Correlative studies include development of patient derived xenografts, evaluation of genomic signatures of resistance and response, and comparison of chemotherapy responses between xenografts and host patients. Five patients' serial tumor samples and germline DNA were studied by exome and transcriptome sequencing. Three of these patients had an additional on-treatment sample at cycle 1 day 3. Two patients lacked residual disease samples- one was not banked and the other could not be accurately genotyped due to low cellularity. The median sequencing depth was 90.13x. Sequencing was performed on either fresh frozen or formalin-fixed paraffin-embedded samples with high cellularity (≥50%). After identifying somatic mutations in each tumor series, we evaluated whether each mutation was persistent, emergent, or cleared by comparing pre- and post-treatment (and when possible, on-treatment) samples.

Results-All five patients had response to neoadjuvant chemotherapy based on caliper-based and pathologic (residual cancer burden I or II) measurements. All residual disease remained TNBC by standard immunohistochemistry and all samples were basal-like from PAM50 gene expression analysis. We identified 908 somatic mutations, including the expected variants in *TP53* which persisted in all post-treatment samples. Non-silent somatic variants were identified in other breast cancer-related genes, including *GATA1*, *FBXO11*, *PIK3R1*, *AXIN2*, *ARID1B*, *BRCA2*, and *RBCC1*. In spite of the clinico-pathologic evidence of response, we observed little change in clonal architecture, as derived from the purity-corrected variant allele fractions between baseline, cycle 1 day 3, and post-chemotherapy samples. Copy number alterations were likewise stable and transcriptional-based assessment indicated that patterns of mutant allele expression in driver genes were retained throughout the course of treatment.

Conclusion-In TNBC patients undergoing neoadjuvant platinum-based chemotherapy, there were no apparent shifts in the prevalence of known breast cancer specific somatic variants during or after chemotherapy. Despite pathologic response, core genomic features appear to be preserved in TNBC patients with residual disease following chemotherapy, likely accounting for high rates of relapse in these patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-12

Title: An exploratory correlative biomarker analysis of NSABP FB-7, a phase II randomized trial evaluating neoadjuvant therapy with weekly paclitaxel (P) plus neratinib (N) or trastuzumab (T) or neratinib and trastuzumab (N+T) followed by doxorubicin and cyclophosphamide (AC) with postoperative T in women with locally advanced HER2-positive breast cancer

Gavin PG G, Kim PS S, Lipchick C, Langley E, Feng H, Meyer GR R, Rim Kim S, Michel JP P, Jacobs SA A, Srinivasan A and Pogue-Geile KL L. NSABP Foundation and DiaTech Oncology.

Body: The NSABP FB-7 trial enrolled HER2-positive breast cancer patients (pts) with locally advanced disease who were randomly assigned to trastuzumab (T) or neratinib (N) or the combination (T+N) with weekly paclitaxel (P) followed by standard AC. A total of 126 pts were randomized to Arm 1 (T+P→AC), Arm 2 (N+P→AC), or Arm 3 (N+T+P→AC). Eligibility criteria included women >18 years of age, ECOG PS 0-1, stage IIB-IIIC invasive breast cancer, HER2-positivity by IHC 3+, FISH, or CISH as determined by local laboratories, hormone receptor positive or negative, LVEF \geq 50%, and adequate laboratory parameters. The primary endpoint was pathologic complete response (pCR) in breast and nodes and was reported at SABCS 2015 (Jacobs et al, abstract # PD5-04). Dual anti-HER2 therapy yielded greater pCR rates than single targeted therapies in the overall cohort, and the magnitude of pCR was highest in HR-negative patients. Since this initial report, an in depth correlative biomarker analysis with clinical outcome has been performed and will be presented.

Methods: In an effort to discover candidate predictive markers of response to study drugs, pre-treatment core biopsy samples (n=59) and post treatment surgical samples (n=17) were obtained from a subset of patients. Samples were collected prospectively from 2011-2014 and stored prior to batch analysis. Retrospective correlation with clinical outcome was performed after datalock of 1st Sept. 2015. Expression and activation of HER family RTKs, including truncated HER2, as well as downstream components of PI3K/AKT and MAPK pathways, were quantified using the multiplexed microarray CEER™ platform (Collaborative Enzyme-Enhanced Reactive immuno-assay). pCR data were available in n=51 pts from the biomarker cohort. After excluding low tumor content non-evaluable samples, correlative biomarker analysis was performed in n=42 patients. Breast cancer subtypes were assessed with RNA-Seq to assess changes in gene expression and subtypes before and after treatment and association of pathways with pCR. The distribution, in our study population, of MammaPrint and Blueprint scores (Agendia) and their association with pCR will be presented. Results from a custom AmpliSeq (Thermo-Fisher) gene-panel used to assess the variant status of 117 genes potentially relevant to the development of resistance to anti-HER2 therapies, will be presented.

Conclusions: Different patterns of baseline expression and activation levels of several key drivers, such as p95HER2, HER2, HER3, and PI3K, were observed between pCR in Arm1 (T+P→AC) and Arm 2 (N+P→AC). In particular, Arm 2 pCR displayed higher baseline levels of truncated HER2 compared to non-pCR. This exploratory marker analysis generates the hypothesis that high p95HER2 levels as measured by CEER™ may predict the likelihood of response to a neratinib containing regimen in the neoadjuvant setting in locally advanced breast cancer. Other correlatives will be discussed.

Support: Puma Biotechnology, Inc.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-13

Title: The mutation detection and a high throughput screening of driver mutations in PI3K/AKT pathway based on next generation sequencing

Chen L, Yang L, Yao L, Hu X and Shao Z. Fudan University Shanghai Cancer Center, Shanghai, China; Cancer Institute, Fudan University Shanghai Cancer Center, Shanghai, China and Shanghai Medical College, Fudan University, Shanghai, China.

Body: Background

The deregulation of the PI3K/AKT signaling pathway is essential to malignant cellular processes of breast cancer, including proliferation, apoptosis, and drug response. Oncogenic activating somatic mutations in the PI3K/AKT pathway are pervasive. However, it remains difficult to discriminate between driver and passenger mutations. This study was conducted to identify the landscape of genetic mutations in the PI3K/AKT pathway using Amplicon Sequencing in a Chinese population. Notably, we developed a Gateway-based mutation barcoding (GaMB) library which enables a high-throughput mutation-phenotype screen for specific vulnerable mutations that contribute to the cancer development and drug resistance.

Method

We collected 149 breast cancer specimens in a Chinese population and performed Ion Torrent Amplicon Sequencing for the key genes in PI3K/AKT pathway: *PIK3CA*, *PIK3R1*, *AKT1*, *AKT2*, *AKT3*, *PTEN*, *PDK1*, and the canonical tumor suppressor gene *TP53*, at 1000× coverage. Next, we established a high-throughput GaMB library that contained all of the *PIK3CA* mutations, either newly identified in Chinese population or reported in TCGA and COSMIC database, and tagged each mutations with a specific barcode. We then applied this library to functional screening processes using proliferation and drug response selection (doxorubicin or BKM-120) assays through which we screened the functional mutations with specific characteristics. The genomic DNA of the pooled surviving cells from the library, as well as the original cells before the screenings, was extracted and used for PCR amplification of the barcode regions, and then detected using Illumina Miseq sequencing to analyze the functional mutations. We then validated the cellular 2D- & 3D- proliferation abilities and the status of PI3K/AKT pathway activation in presence of identified mutations, respectively.

Result

Mutations in the *PIK3CA* (44%), *PIK3R1* (37%), *AKT3* (15%) and *PTEN* (12%) genes were the most prevalent. Mutations in *PIK3CA* were present in 65 samples (43.6%) which is similar to that reported in TCGA database. *PIK3R1* (37%) was found significantly mutated, with a novel recurrent mutation, N595S, being identified in 24 patients. Similarly, *AKT1* (10.1%), *AKT2* (10.1%), and *AKT3* (14.8%) mutations were present at a higher frequency in our population than has been reported in the TCGA and COSMIC database.

In the *PIK3CA*-GaMB library, our highest-ranking mutations included the previously validated deleterious mutations H1047R and E545K and several mutations of uncertain significance, including E39K, G1049R, N345I, N345K, M1043V, and H1047T. In the validation assays, we found a high phenotype-consistency of these identified mutations using these functional validation techniques. The breast cancer cells harboring identified mutations exhibit a relatively higher proliferation ability and tolerance to chemotherapy and pathway inhibitors.

Conclusion

This study identified the landscape of genetic mutations in the PI3K/AKT pathway using Amplicon Sequencing in a Chinese population. A novel developed GaMB screening platform may allow the rapid identification of significant mutations that dominate breast cancer development and drug responses during treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-14

Title: Rates of immune infiltration in patients with triple-negative breast cancers by molecular subtype and in patients with inflammatory and non-inflammatory breast cancers

Harano K, Wang Y, Lim B, Seitz RS S, Morris SW W, Bailey DB B, Hout DR R, Skelton RL L, Ring BZ Z, Masuda H, Rao AUK UK, Woodward WA A, Reuben JM M and Ueno NT T. Morgan Welch Inflammatory Breast Cancer Research Program and Clinic, The University of Texas MD Anderson Cancer Center, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX; Insight Genetics, Inc., Nashville, TN and Showa University, Shinagawa, Tokyo, Japan.

Body: *Background*

In patients with triple-negative breast cancer (TNBC), tumor-infiltrating lymphocytes (TILs) have been reported to be associated with improved survival. Lehmann et al. identified 6 molecular subtypes of TNBC [basal-like (BL) 1, BL2, mesenchymal (M), mesenchymal stem like (MSL), immunomodulatory (IM), and luminal androgen receptor (LAR)], and we previously reported that TNBC subtype is a predictor of pathologic complete response (pCR). Recently, the IM gene expression signature has been shown to be indicative of the presence of TILs and has been incorporated into TNBC subtyping as a modifier of the other groups rather than a separate subtype. However, the association between TNBC subtype and the presence of TILs is not known. We hypothesized that the BL2 and LAR subtypes, which have low pCR rates, have low rates of immune infiltration. Inflammatory breast cancer (IBC) is an aggressive cancer that is frequently triple-negative. The association between IBC and the presence of TILs also is not known. In this study, we analyzed the association between TNBC molecular subtype and the IM signature and determined whether the IM signature differed between patients with IBC and non-IBC.

Methods

We retrospectively analyzed 88 patients with TNBC from the World IBC Consortium dataset for whom IBC status was known (IBC, n=39; non-IBC, n=49) and tumor gene expression data were available. TNBC specimens were classified using the TNBCtype algorithm (Insight Genetics, Inc., TN, USA), which uses a 101-gene signature. For each tumor, the *TNBCtype* algorithm reports the TNBC molecular subtype (BL1, BL2, M, MSL, or LAR) and the IM status, which is described as positive (IM+) or negative (IM-). Recently, Fisher's exact test was used to analyze differences in subtype distribution between the IM+ and IM- tumors.

Results

The subtype distribution differed significantly between the IM+ and IM- tumors

IM signature in TNBC subtypes

Subtype	Total (n=88)	IM+ (n=32)	IM- (n=56)
BL1	30	15 (50)	15 (50)
BL2	2	0	2 (100)
M	8	0	8 (100)
MSL	31	13 (42)	18 (58)
LAR	12	1 (8)	11 (92)
Not determined	5	3 (60)	2 (40)

($p=0.0087$). The majority of IM+ cases occurred in the BL1 and MSL subtypes. No IM+ cases were observed in the BL2 or M subtypes, and only 1 was observed in the LAR subtype. IM+ cases occurred at roughly the same frequency in patients with IBC (33%) and non-IBC (37%, $p=0.73$).

Conclusions

TNBC molecular subtypes differ in their degree of immune infiltration, and most IM+ TNBCs are of the BL1 and MSL subtypes. Our finding that the proportion of IM+ cases was not different between IBC and non-IBC indicates that TILs are recruited to the

tumor microenvironment similarly in IBC and non-IBC tumors. Further, Pietenpol et al recently showed that the MSL signature represents normal stromal cells rather than tumor cells by performing laser-capture microdissection of TNBC specimen. Validation studies are needed to corroborate and further expand upon our findings.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-15

Title: Detection of molecular alterations in breast cancer through next generation sequencing of both tumor tissue and circulating tumor DNA: The UC San Diego Moores Cancer Center experience

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Body: Background: Next generation sequencing (NGS) analysis of actionable molecular alterations has the potential to guide cancer treatment, especially for patients with advanced cancer who have progressed on standard treatment. In this study, we analyzed tumor biopsies and peripheral blood from 62 patients with advanced breast cancer by two different NGS clinical-grade assays for molecular alterations in tumor tissue or in circulating tumor DNA (ctDNA). We used these results to determine if these specimens have potentially “actionable” alterations that could guide cancer therapy.

Methods: From 2014 to 2016, 62 patients with advanced breast cancer had plasma sent for ctDNA analysis (Guardant360 assay; 54 to 70 genes) Thirty-eight of these patients (61%) also had tumor biopsies evaluated by NGS (FoundationOne®; 182 to 315 genes). Alterations were defined as mutations, insertions, deletions, truncations, or rearrangements or amplifications/copy number variations. Patients that harbored multiple alterations in the same gene were not counted as having separate alterations; however, if a gene amplification and an alteration were found in the same gene these were counted as separate events. Variants of unknown significance (VUS) and synonymous mutations were excluded from both assays. Data were collected and analyzed according to a UCSD Institutional Review Board approved protocol.

Results: The median age of our patients at the time of ctDNA analysis was 55 years (range, 44 to 84 years); the median age at the time of tissue biopsy for NGS was 52 years (range, 39 to 82 years). One patient was male. The most common receptor status was estrogen receptor (ER) and progesterone receptor (PR) positive, human epidermal growth factor receptor 2 (HER2) non-amplified or negative (neg)(N=44; 71%), followed by triple negative breast cancer (ERnegPRnegHer2neg) (N=10, 16%), triple positive (N=6, 1%) and finally ERnegPRnegHER2positive (N=2, 0.03%). One patient of 38 (2%) had no tumor alteration detected and 19 of 62 had no ctDNA alterations (31%). In 38 breast cancer patients with tumor NGS results, alterations were detected in 79 unique genes, with the most frequent being *TP53* (37% of patients), *PIK3CA* (24%) and *GATA3* (24%) genes. In the 62 patients with ctDNA analysis, 31 unique genes had at least one alteration, with the most frequent being *TP53* (36% of patients) and *PIK3CA* (23%) and *EGFR* amplification (11%) (*GATA3* was not analyzed in the ctDNA assay). Both assays had a high rate of detection for potentially actionable mutations: 41 out of 62 patients (66%) by ctDNA and 34 out of 38 (89%) by tumor NGS. No two patients harbored identical genomic profiles by either tumor NGS or plasma ctDNA analysis except for 1 patient who had no alterations detected by either assay.

Conclusions: Plasma and tissue NGS analysis appear to be complementary assays that yield a high percentage of potentially actionable alterations in patients with advanced breast cancer. Studies of the clinical impact of NGS-guided therapy in breast cancer are warranted.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-16

Title: Multi-level gene expression signatures provide significant prognostic information in metastatic breast cancer patients

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Body: Background: We have previously demonstrated how transcriptional pathway activity and the molecular subtypes of breast cancer metastases significantly influence patient post-relapse survival. Here we extend our analysis to determine whether the prognostic information provided by gene expression signatures in primary breast tumours is also relevant in the metastatic setting. Specifically, we test the research versions of the Genomic Grade Index (GGI), Mammaprint, Recurrence score (RS) and PAM50 gene signatures along with our own cell-cycle based classifier (CCS).

Methods: 287 patients with morphologically confirmed loco-regional or distant breast cancer relapse were enrolled in the Swedish multicenter TEX trial from December 2002 until June 2007. Of these, sufficient tumour RNA for gene expression profiling was obtained from metastatic tissue by fine needle aspiration from 111 patients (totalling 120 relapse biopsies). Gene signatures were applied as described in the original research articles and their relationship to short (1.5 year) and long-term (5 year) post-relapse survival was assessed using likelihood ratio, Kaplan-Meier and Cox regression analysis.

Results: As anticipated from an aggressive metastatic cohort, the majority of samples (> 70%) were classified into intermediate or high risk groups by all signatures. In both short and long-term survival analysis only PAM50 provided statistically significant prognostic information (short: $LR\chi^2 = 14.7$, $p = 0.005$ and long: $LR\chi^2 = 13.2$, $p = 0.010$), with the cell cycle score signature displaying a prognostic trend in long-term survival only ($LR\chi^2 = 5.2$, $p = 0.074$). Kaplan-Meier curves and Cox regression analysis suggest that the strength of both signatures resides in their ability to select a group of low-risk patients with better long-term survival.

Conclusions: Our findings demonstrate the prognostic utility of the multi-level PAM50 and to a lesser extent, cell cycle score signatures in predicting survival of patients with metastatic breast cancer. Simpler binary gene expression signatures (GGI and Mammaprint) do not appear to capture the same prognostic information and as such may have limited utility in a metastatic setting.

Short and long term survival Likelihood Ratios for five gene expression signatures in metastatic breast cancer

Gene Signature	Short term survival (1.5 year)		Long term survival (5 year)	
	$LR\chi^2$	P-value	$LR\chi^2$	P-value
GGI	1.3	0.251	0.5	0.500
Mammaprint	1.7	0.190	0.6	0.427
RS	3.9	0.143	4.4	0.110
CCS	2.8	0.242	5.2	0.074
PAM50	14.7	0.005	13.2	0.010

GGI: Genomic grade index; RS: Recurrence score; CCS: Cell cycle score

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-07-17

Title: The SCAN-B study: 5-year summary of a large-scale population-based prospective breast cancer translational genomics platform covering a wide geography of Sweden (NCT02306096)

Saal LH H, Hegardt C, Vallon-Christersson J, Häkkinen J, Ehinger A, Manjer J, Larsson C, Loman N, Rydén L, Malmberg M and Borg Å. Lund University, Lund, Sweden; Blekinge Country Hospital, Karlskrona, Sweden; Skåne University Hospital, Malmö, Sweden; Lund University, Lund, Sweden; Skåne University Hospital, Lund, Sweden and Skåne University Hospital, Lund, Sweden.

Body: Background:

Breast cancer exhibits significant molecular, pathological, and clinical heterogeneity. Current clinicopathological evaluation is imperfect for predicting outcome, which results in overtreatment for many patients, and for others, leads to death from recurrent disease. Therefore, additional criteria are needed to better personalize care and maximize treatment effectiveness and survival. To address these challenges, large-scale population-based studies are needed to develop and evaluate new predictive biomarker tests under real-world conditions.

Methods:

In 2010 we initiated the Sweden Cancerome Analysis Network - Breast (SCAN-B) multicenter prospective study (ClinicalTrials.gov identifier NCT02306096) with long-sighted aims to 1) analyze breast cancers with next-generation genomic technologies for translational research in a population-based manner and integrated with healthcare; 2) decipher fundamental tumor biology from these analyses; 3) utilize genomic data to develop and validate new clinically-actionable biomarker assays; and 4) establish real-time clinical implementation of molecular diagnostic and treatment-predictive tests. Eligibility criteria are suspicion or confirmed diagnosis of primary breast cancer. Eligibility will be extended to recurrent breast cancer in late 2016. For all patients, tumor biopsy and/or surgical tumor specimen and baseline blood samples are collected, as well as follow-up blood samples at defined intervals, and clinical data are obtained from regional and national databases. From all samples, DNA, RNA, and protein fractions are isolated, and tissue arrays are constructed. In the first phase, we focus on molecular profiling of tumor tissue by next-generation RNA-sequencing.

Results:

From August 2010 through May 2016, we have consented and enrolled 8,669 patients with primary breast cancer at 9 hospital sites in Sweden, representing approximately 85% of eligible patients in the catchment area. Preoperative blood samples have been collected for 8,288 (96%) patients and primary fresh-frozen tumor specimens collected for 6,129 (71%) patients. All tumors have been RNA-sequenced, and newly enrolled cases are analyzed in “real-time” within an average of 7 days after biopsy/surgery. Herein we describe the study infrastructure and protocols and present initial proof of concept results from prospective RNA-sequencing including tumor molecular subtyping, detection of driver gene mutations, and determination of ER, PgR, HER2, Ki67, and tumor grade from RNA-seq data. Prospective patient enrollment is ongoing and pilot clinical reports are being evaluated at multidisciplinary breast cancer conferences.

Conclusions:

We demonstrate that population-based collection and real-time RNA-sequencing analysis of breast cancer is feasible at large-scale. The SCAN-B Study should significantly reduce the time to discovery, validation, and clinical implementation of novel molecular diagnostic and predictive tests. We welcome the participation of additional comprehensive cancer treatment centers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-08-01

Title: Regulation of estrogen receptor- α by NF1

Zheng Z-Y, Cakar B, Lavere P, Cao J, Yao J, Singh P, Lei JT T, Toonen JA A, Haricharan S, Anurag M, Shah K, Kavuri M, Chan DW W, Chen X, Gutmann DH H, Foulds CE E, Ellis MJ J and Chang EC C. Baylor College of Medicine, Houston, TX and Washington University in St. Louis, St. Louis, MO.

Body: Background. Although great strides have been made in targeting the ER pathway for treating ER⁺ breast cancer, relapse and death is common and is closely linked to resistance to ER-targeting agents. As a result, the majority of deaths from breast cancer still come from ER⁺ tumors. To discover drivers for endocrine resistance, we have sequenced tumor DNAs from a cohort of >600 patients treated with 5-year tamoxifen (Tam) monotherapy with a median 10.4 years follow up. Our preliminary data show that the worst outcome mutations (Hazard Ratio of ~3 for relapse) were mostly those of the *Neurofibromatosis type 1 (NF1)* gene (encoding Neurofibromin), with nonsense/frame shift mutations creating early stop codons.

Germline *NF1* mutations cause neurofibromatosis type 1, a common inherited disorder that predisposes individuals to both benign and malignant tumors of the nervous system, as well as an increased risk for breast cancer. Analysis of DNA sequencing data has also shown that the *NF1* gene is mutated in a wide range of common cancers (e.g., melanoma, lymphoma, and cancers of the lung, breast, and colon). Thus, *NF1*-deficiency underlies the formation and/or progression of a large number of cancers, so that the development of therapies targeted to *NF1*-deficient malignancies would have broad impact.

These observations support the hypothesis that *NF1* gene inactivation is associated with aggressive tumor behaviors, such as endocrine therapy resistance in breast cancer. The key focus of this study is to define how the *NF1* protein neurofibromin, regulates endocrine therapy resistance. Although neurofibromin is best known as a negative regulator for Ras, our data show that it may have other functions.

Method. Our data suggest that many of the identified nonsense/frame shift create a *NF1* null state; thus, we have used gene-silencing to recapitulate the effects of such *NF1* mutations on the activities of ER⁺ breast cancer cells. *NF1*⁺ and *NF1*⁻ ER⁺ breast cancer cells were grown in defined media to measure how estradiol (E₂) and Tam impact their growth, transforming activities, and gene expression. The binding between neurofibromin and components of the ER transcriptional pathway was measured biochemically and using the mammalian two-hybrid system.

Results. Our data showed that *NF1*-silenced cells use Tam as an agonist and can grow with very little E₂, and these activities are driven by enhanced recruitment of ER to the ERE, leading to efficient expression of many classic ER-responsive genes. Expressing the NF1-GAP domain does not restore normal responses to Tam and E₂ in *NF1*-silenced cells, suggesting that neurofibromin can regulate ER activity in a Ras-independent manner. To investigate the possibility that neurofibromin can directly regulate ER, we found that it can bind ER; furthermore, neurofibromin was more strongly recruited to the ERE by Tam than by E₂.

Conclusion. Our data support a model whereby neurofibromin acts like a co-repressor for ER. As such, *NF1* loss may result in more aggressive tumor behaviors by activating, not only the Ras pathways, but also the ER transcriptional pathways. Simultaneous activation of two powerful oncogenic pathways by the loss of a single tumor suppressor may explain why neurofibromin is such a potent tumor suppressor lost in a wide range of cancers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-08-02

Title: Mutant GATA3 actively promotes the growth of normal and malignant mammary cells

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Body: GATA3 is a transcription factor expressed in luminal breast epithelial cells and is required for mammary gland development. Analysis of TCGA data reveals that somatic heterozygous mutations in GATA3 occur in up to 15% of estrogen receptor positive breast tumors, and that these tumors are diagnosed a median of eight years earlier than other estrogen receptor positive tumors, suggesting a more aggressive phenotype. These mutants have been proposed to be null alleles resulting in haploinsufficiency, however the mutation spectrum of GATA3 in breast cancer is in sharp contrast to that found in HDR syndrome, a true GATA3 haploinsufficiency disease. Based on this disparity, we propose that there is a selective pressure to mutate and retain a portion of the GATA3 in breast cancer. Here we focus on the GATA3 mutants which lack the second zinc finger which is responsible for GATA motif binding. Expression of these mutants accelerated xenograft tumor growth by ZR751 cells, and transgenic expression in mouse mammary glands promoted precocious lobuloalveolar development. We have used integrated gene expression and CHIP-Seq profiling to demonstrate that these zinc-finger deleted proteins retain the ability to associate with the genome by tethering to complexes associated with FOXA1 and AP-2gamma recognition motifs, where they modulate the expression of adjacent genes. These data support a model in which the GATA3 mutations recently observed in breast cancer encode for active transcription factors which elicit proliferative phenotypes in normal mammary epithelium and promote the growth of estrogen receptor positive breast cancer cell lines.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-08-03

Title: Identification and characterization of a novel endoxifen substrate, PKC β 1, and its interaction with the estrogen receptor

Guo C, Kuffel MJ J, Kudgus RA A, Huang Z, Bode AM M, Cheng J, Suman VJ J, Reid JM M, Bruinsma ES S, Subramaniam M, Ames MM M, Hawse JR R and Goetz MP P. Mayo Clinic, Rochester, MN and The Hormel Institute, University of Minnesota, Austin, MN.

Body: Background: The primary mechanism by which tamoxifen (Tam) and its metabolites exert their biologic effects is through estrogen receptor (ER) binding and inhibition of ER signaling. We and others demonstrated that endoxifen (Endx) has greater antitumor activity *in vitro* and *in vivo* compared to Tam and the first-in-human Endx phase I study demonstrated its antitumor activity in patients with prior progression on Tam (Goetz SABC 2015). PKCs are a family of serine/threonine-specific protein kinases that regulate signaling pathways involved in cell proliferation and tumorigenic transformation. Our prior protein docking studies suggested endoxifen may be a substrate for PKCs. Here we report the effects of Tam and Endx on PKC β 1 binding, kinase activity, as well as interactions between PKC β 1 and ER α .

Methods: Surface Plasmon Resonance (SPR, Biacore T200, GE Healthcare) was used to evaluate binding of Tam, N-desmethyl Tam (NDMT), 4-HT, and Endx to PKC β 1 and PKC β 2. The effects of Tam and Endx on PKC β 1 kinase activity were determined. Proliferation and colony formation in MCF7 parental and PKC β 1 overexpressing cells were evaluated. siRNA silencing was used to knockdown PKC β 1 expression in the following cells: MCF7 aromatase expressing cells that were either sensitive (MCF7/AC1) or resistant to letrozole (MCF7/AC1 L-resistant); T47D; and MDA-MB-361. Coimmunoprecipitation assay and DUOLink *in situ* proximity ligation were used to investigate the interaction between PKC β 1 and ER α .

Results: Endx more potently inhibited PKC β 1 kinase activity compared to Tam (IC50 350 nM vs 47.8 μ M) with K_D s for PKC β 1 binding as follows: Endx (100 nM), Tam (2 μ M), 4-HT (2 μ M) and NDMT (> 7 μ M). None of the SERMs exhibited PKC β 2 binding. In the MCF7/AC1 and MCF7/AC1 L-resistant cells, PKC β 1 knockdown resulted in ER α degradation and potently inhibited cell proliferation. These results were confirmed in T47D and MDA-MB-361 cells. Notably, PKC β 1 knockdown in MCF7/AC1 cells resulted in significantly greater E₂ induced proliferation comparing siRNA knockdown vs. control. To further explore these effects, we evaluated the effects of PKC β 1 overexpression in MCF7 cells and demonstrated that PKC β 1 overexpression reduced cell proliferation and colony formation compared to parental MCF-7 cells without affecting ER α protein stability.

Coimmunoprecipitation assays in transiently transfected MCF-7 cells with exogenous PKC β 1 as well as PKC β 1 expressing MDA-MB-231 cells transiently or stably transfected with ER α demonstrated PKC β 1 and ER α interaction, with confirmation by Duolink assay that this interaction occurs in the cytoplasm.

Conclusions: Our findings demonstrated that endoxifen binds and inhibits PKC β 1 at relevant concentrations achieved in the endoxifen clinical trial studies. PKC β 1 interacts with cytoplasmic ER α and PKC β 1 knockdown inhibits cell proliferation and enhances ER α turnover. However, in PKC β 1 overexpressing cells, PKC β 1 may exhibit tumor suppressive effects. These data suggest a complex interaction between PKC β 1 and ER α and that endoxifen's effects on PKC β 1 may alter drug response of endocrine therapy. Further studies are ongoing to characterize the role of PKC β 1 and its role in ER biology and response to endoxifen.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-08-04

Title: SOX9 is a critical regulator of triple-negative breast cancer cell growth and invasion

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Body: Background: SRY (Sex Determining Region Y)-related HMG-box (SOX) genes belong to a super-family of genes, which is characterized by a homologous sequence called the HMG-box residing on the Y-chromosome. There are 20 SOX genes present in humans and mice. We performed a siRNA screen of SOX transcription factors, and found that SOX9 was essential for breast cancer cell growth. The SOX9 protein recognizes the sequence CCTTGAG along with other members of the HMG-box class DNA-binding proteins and has been shown to be required for development, differentiation and lineage commitment. Moreover, SOX9 is expressed in adenocarcinomas, and is highly expressed in the most aggressive cancers. Our previous data shows SOX9 is highly expressed in “triple negative breast cancer” (TNBC) than in non-TNBC. Thus, we hypothesized that the SOX9 transcription factor acts as an essential molecule regulating TNBC growth and invasion. To test the hypothesis, we used SOX9-overexpressed, or SOX9-knockdown/knockout breast cancer cell models to determine whether SOX9 is necessary and/or sufficient to regulate TNBC cell proliferation, migration and invasion.

Methods: We measured the cell growth using an automated cell counting assay. Cell migration and invasion were detected by transwell migration & invasion assays in ER-positive (MCF7 and ZR75-1) and ER-negative (MDA231 and MDA468) breast cancer cells. DOX-inducible SOX9-knockout cell lines were established in MDA231, MDA468, and LM2 cell lines using an inducible Cas9-CRISPR system. A SOX9 expressing lentivirus was used to overexpress SOX9, and siRNAs was used to knockdown SOXs in the different breast cancer cells. Protein and mRNA levels of SOX9 in TNBC, non-TNBC, immortalized human breast epithelial cell lines were examined by western blotting and qRT-PCR assay.

Results: Knockdown of SOXs by siRNA caused decreased cell proliferation of MDA231 by $\geq 50\%$ and of MDA468 by 30%-50% in siSOX4, siSOX6, siSOX9, siSOX10 and siSOX11 treatment groups (but not in siSOX8 and siSOX17 treatment groups). However, in MCF7 and T47D cell lines, treatment with siRNA to these SOX factors did not cause significant cell growth reduction. We demonstrated that SOX9 is more highly expressed in TNBC cells at both the mRNA and protein levels. Knockdown of SOX9 decreased cell migration and invasion of MDA231 to 25% and 50% respectively. The same effect also was observed in MDA468 cells, with approximately a 50% decline in migration and invasion. In SOX9-knockout MDA231, MDA468, and LM2 cells, cell proliferation, migration, and invasion were significantly reduced. In contrast, overexpression of SOX9 in MCF7 and ZR75-1 cells increased cell migration and invasion. We are now conducting *in vivo* studies to determine the effect of SOX9 on breast cancer cell metastasis.

Conclusion: SOX9 is a critical regulator of TNBC cell proliferation, migration and invasion. These studies suggest that regulating SOX9 transcription factor and its signaling pathway will be a promising therapeutic strategy to treat TNBC and prevent metastasis.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P1-08-05

Title: Discovering drug targets for aggressive breast cancer with *TP53* missense mutations by a genome-wide screen

Pal A, Park J, Gonzalez-Malerva L, Eaton S and LaBaer J. The Biodesign Institute, ASU, Tempe, AZ.

Body: Breast cancer is a heterogeneous disease and has subtypes with distinct phenotypic and molecular characteristics. Genetically, 30% of all breast tumors and 80% of the basal-like breast cancer (BLBC) subtype harbor mutations in *TP53* and numerous sporadic somatic mutations in other genes. This molecular heterogeneity has posed a challenge in developing safe and effective therapeutic regimens to treat a broad spectrum of breast cancer patients, and discovery of intra-subtype along with inter-subtype heterogeneity has added another layer of complexity for targeted therapies. Based on the analysis of somatic mutation profiles in the BLBC subtype, we identified a wide variety of *TP53* missense mutations and thousands of co-existing mutations, which led us to hypothesize that intra-subtype heterogeneity is derived from combinatorial effects of neo-morphic (gain-of-function) activities of different types of missense mutant p53 proteins and complex interplay between specific *driver* mutations in *TP53* and a distinct subset of functionally important co-mutations (or “*co-drivers*”). We ectopically overexpressed the ten most prevalent missense mutations in *TP53* found in breast cancer tumors in non-transformed mammary epithelial cells and examined their cellular functions associated with the hallmarks of cancer. The results showed widely different spectrums of phenotypic changes in cell proliferation, resistance to apoptosis, cell invasion, anoikis resistance and cell polarity. As a proof of concept for the 'co-drivers', we knocked-out *PTEN* in a non-invasive *TP53* mutant cell using the CRISPR and shRNA systems. Inactivation of *PTEN* in non-invasive *TP53*-mutant cell increased its invasiveness. *TP53* mutations and *PTEN* deletions are frequent in BLBC patients, who have significantly higher rates of metastasis, recurrence and a lower survival rate than the other subtypes. The absence of three signaling hormone receptors, which can be targeted by specific inhibitors, significantly limits available therapies for the majority of BLBC patients to surgery and cytotoxic chemo/radiotherapies. Combination therapies targeting the *driver* and *co-driver* gene associated pathways could be quite promising solutions to treat aggressive breast cancer cases. To address this, we have developed a genome-wide CRISPR based gene knock-out screening approach to test the effect of loss-of-function of individual human genes in clinically important *TP53* mutant backgrounds that can promote cancer-like behaviors and identify the combinations of '*TP53* mutation and co-driver pathways' that can be targeted by specific inhibitors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-08-06

Title: BC200 lncRNA is involved in the progression of triple negative breast cancer

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Body: Long non coding RNAs (lncRNAs) have been identified as regulators of the cell cycle, apoptosis, and DNA damage among other processes that if deregulated, may lead to cancer by acting as proto-oncogenes, tumor suppressor genes, and drivers of metastatic transformation. Using RNA sequencing we have identified 42 differentially expressed lncRNAs from a healthy cohort of parous vs. nulliparous women. After bioinformatics and RT-qPCR analysis, we have focused on a vaguely studied lncRNA called BC200 that is highly expressed in the nulliparous postmenopausal breast tissue. It is known that BC200 lncRNA is overexpressed in invasive and pre-invasive breast cancer; however, its functional role in the initiation and progression of breast cancer is poorly understood. In the present work we provide insight on the role of BC200 in the context of luminal and triple negative breast cancer (TNBC). We have confirmed that BC200 is highly expressed in breast cancer tissue and in widely used breast cancer cell lines such as MCF7, T47D, MDAMB231, and Hs578T. Using a lentiviral system we successfully obtained cell lines which stably express BC200. Overexpression of BC200 increases proliferation, migration, and invasion potential *in vitro* in the cell lines tested, specifically luminal T47D and TNBC MDAMB231. Xenograft studies performed in the mammary fat pad of female SCID mice confirm the role of BC200 as a tumor promoter. Tumors in mice injected with MDAMB231 cells overexpressing BC200 were 4.5 times bigger than tumors in the control group in only 6 weeks when injecting 1 million cells. Moreover, we have determined, using reverse transcriptase PCR targeting genes less than 200 kb from the start site of BC200, that when BC200 is overexpressed, CALM2 is downregulated in both T47D and MDAMB231 cell lines. CALM2 or Calmodulin is a calcium binding protein that plays a role in signaling pathways, cell cycle progression, proliferation, and apoptosis. Mutations in CALM2 are associated with increased risk of breast cancer. Our positive results on *Cis* regulation are being expanded using chromatin isolation by RNA immunoprecipitation to determine BC200's genome wide regulation.

These results demonstrate the participation of BC200 lncRNA in the progression of breast cancer. Notably, BC200 regulates nearby genes that have an implication in cancer progression. BC200, identified in the normal breast tissue of nulliparous women, not only plays a key role in breast cancer progression but also provides a new insight in the preventive role of pregnancy by the downregulation of the expression of this lncRNA in the normal parous breast. *[This work was supported by the NCI (National Cancer Institute) Core Grant CA06927 to Fox Chase Cancer Center and generous support from Christian - Diane Martin, the Flyers Wives, and Joseph - Barbara Breitman to Dr. J. Russo, MD].*

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-08-07

Title: Assessing the impact of loss of NF1 protein on endocrine therapy resistance

Cakar B, Chan D, Yan P, Zheng Z, Singh P, Lei JT Thomas, Haricharan S, Ellis M and Chang E. Baylor College of Medicine, Houston, TX.

Body: Background: The vast majority of breast cancers belong to the luminal subtype, which expresses the estrogen receptor- α (ER). Although great strides have been made in targeting the ER pathway for treating the ER⁺ tumors, relapse and death is common and ongoing. In order to identify the cause for treatment resistance, we have conducted a retrospective analyses on the tumor genomes of >600 patients treated by tamoxifen monotherapy in the adjuvant setting with a median follow-up of 10.4 years. Our data have revealed that *NF1* (*Neurofibromatosis type 1*) gene loss of function mutations were greatly associated with poor prognosis. NF1 is a tumor suppressor acting mostly as a GAP (GTPase activating protein) to switch off activated Ras. We aim to define the impact of loss of NF1 protein on patient outcome in ER⁺ breast cancer patients by establishing an immunohistochemistry (IHC) protocol to detect NF1.

Method and results: We have first surveyed commercially available antibodies by Western blot and found one that could efficiently detect endogenous NF1. We then use this to validate inducible shRNA clones against *NF1*, as well as a breast cancer cell line that is NF1-null. This antibody has high background. We have thus partially purified a commercially available NF1 antibody by preclearing using NF1-null cell lysate. We then performed immunostaining using NF1-silenced and null cells as control and found that NF1 is mostly cytoplasmic and nuclear. To get antibody of high quality, we have decided to make our own antibody by expressing a C-terminal fragment of *NF1* as a GST-tagged protein (GST-*NF1c*). Production of polyclonal and monoclonal antibody is in progress.

Conclusion: Our clinical profiling data suggest that loss of NF1 protein, a very common event in a wide range of other cancers, promotes endocrine therapy resistance. An efficient IHC protocol will enable us to firmly validate whether loss of the NF1 protein indeed correlates with poor patient outcome. This method will ultimately enable us to identify high risk NF1 deficient patients and to properly treat them.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-08-08

Title: Identification of tumor-promoting and tumor-inhibiting genes with age dependent expression in breast cancer patients

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Body: Age is the number one risk factor for breast cancer development. The breast cancer incidence rate increases with age, following beta distribution, which is approximately linear in range from 30 to 70 years old ^[1]. Transcriptome alterations have been shown to promote tumorigenesis for many types of cancers. Therefore, we hypothesize that the genes with altered expression during aging may promote breast cancer development and/or progression. Using The Cancer Genome Atlas data, we extracted whole transcriptome profiling data of matched normal tissues from 82 female patients with age at diagnosis and menopausal status available. First, we applied simple linear regression to study the association between gene expression level and age at diagnosis on all the 82 patients. 258 upregulated and 240 downregulated genes are identified, which are associated with age. Secondly, 493 upregulated and 254 downregulated genes are identified that are altered by menopause by comparing post-menopausal to pre-menopausal patients (FDR < 0.05). Exclusion of these menopause affected genes from those genes affected by age (258 upregulated and 240 downregulated) results in 148 upregulated and 189 downregulated genes during aging. Thirdly, by comparing matched tumor and adjacent normal samples, 3356 upregulated and 3124 downregulated genes are found to be associated with tumorigenesis. Overlapping of tumorigenesis associated genes with age dependent genes result in 14 upregulated and 24 downregulated genes that are both age and tumorigenesis associated. This discovery is being validated with normal breast tissues from donors in GTEx cohort. Experimental manipulation of the upregulated genes in seven breast cancer cell lines, representing five subtypes of breast cancer, demonstrated their essential role in promoting tumor malignancy, suggesting the upregulation is not merely passenger event. In a large mixed cohort, this panel of genes have significant predictive value in relapse free survival of breast cancer patients.

Funding: CPRIT Research Training Award (RP140105); NIH R01CA192564

Reference:

Francesco Pompei and Richard Wilson (2001). Age Distribution of Cancer: The incidence Turnover at Old Age. Human and Ecological Risk Assessment: Vol. 7, No. 6, pp. 1619-1650.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-08-09

Title: Aurora kinase-A protein stability is negatively regulated by eEF1 α 2 and PTEN in breast cancer: Prognostic and therapeutic implications

Treekitkarnmongkol W, Kai K, Katayama H, Tian W, Rodriguez-Canales J, Sahin AA A and Sen S. The University of Texas M.D. Anderson Cancer Center, Houston, TX and Okayama University, Okayama, Japan.

Body: Background: The *AURKA* gene encoding Aurora kinase-A (Aurora-A) protein is localized on chromosome 20q13 that is frequently amplified and overexpressed across multiple cancer types correlating with patient prognosis. Aurora-A plays a pivotal role in faithful segregation of chromosomes and normal progression of mitosis, peaking at G2/M followed by degradation at the end of mitosis by APC/C (Cdh1). However, regulation of Aurora-A protein stability in human cancer cells is not well elucidated. Here, we show that Aurora-A is targeted for ubiquitination and degradation by SCF complex involving eEF1 α 2 and PTEN in human breast cancer cells. **Methods:** Using a panel of breast cancer cell lines, as *in vitro* models, the eEF1 α 2 was knocked down or ectopically expressed to test the stability of Aurora-A protein. For *in vivo* models, tissue micro arrays of human breast cancer were immunostained for Aurora-A and eEF1 α 2 expression and categorized values were statistically tested by Chi-squared test. In addition, the public breast cancer dataset (Transbig) was used to predict breast cancer prognosis by Kaplan-Meier survival analysis. **Results:** In breast cancer cell lines and patient samples, an eEF1 α 2 non-expressing group showed a trend of higher Aurora-A expression than eEF1 α 2 expressing group, whose trend was significant in patient samples ($P < 0.05$). Knocking down of eEF1 α 2 enhanced Aurora-A protein stability. In contrast, ectopic expression of eEF1 α 2 dramatically decreased Aurora-A protein stability. Inhibition of proteasome activity by MG132 could restore the Aurora-A protein in eEF1 α 2 expressing cells. Biochemical assays showed the direct binding between eEF1 α 2 and Aurora-A, and eEF1 α 2 dependent ubiquitination of Aurora-A. Inverse correlation of the expression levels of the two proteins was also observed through the cell cycle, with eEF1 α 2 levels being high from G1 through G2 phases while Aurora-A expression peaked from G2/M phase through cytokinesis. Taken together, these findings highlight eEF1 α 2 as a novel negative regulator destabilizing Aurora-A through ubiquitin-proteasome proteolytic pathway. Further, mechanistic studies revealed that eEF1 α 2 enhanced the interaction of SCF E3 ubiquitin ligase complex protein; FBXW7 and Cul1, with Aurora-A. In line with this scenario, knocking down of Cul1 increased Aurora-A level. Since PTEN loss was reported to stabilize Aurora-A through inhibiting SCF complex, we tested the significance of PTEN loss in our model. Knocking down of PTEN further stabilized Aurora-A suggesting an independent role of PTEN from eEF1 α 2 in destabilizing Aurora-A. When eEF1 α 2 expressing cells were treated with AKT inhibitor, Aurora-A was destabilized with enhanced bindings between Aurora-A and FBXW7/Cul1. Lastly, low PTEN expression correlated with poor prognosis of Aurora-A over expressing breast cancer patients ($P < 0.01$). **Conclusions:** Aurora-A overexpression in human breast cancer cells may be associated with loss of eEF1 α 2 and PTEN due to reduced interaction of SCF with Aurora-A. Findings indicate significant prognostic and therapeutic implications of altered expression of eEF1 α 2/PTEN/Aurora-A pathways among Aurora-A subset of breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-08-10

Title: Introduction of H1047R oncogenic mutation of PI3K p110alpha subunit in HER2-overexpressing mammary epithelial cells confers a "stem-like" phenotype and acute sensitivity to HSP90 inhibition

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Body: The *Human Epidermal Growth Factor Receptor 2 (HER2)* oncogene is amplified in one-fifth of breast cancers (BC). However, development of resistance against standard anti-HER2 therapies poses a major clinical challenge. Anti-tumor efficacy of HER2-targeting agents depends on inhibition of the downstream phosphatidylinositol-3 kinase (PI3K) signaling cascade. Gain-of-function somatic mutations in the gene encoding the PI3K catalytic subunit p110alpha (*PIK3CA*), co-expressed in about 40% of HER2+ BC, have been implicated in conferring resistance to HER2 monoclonal antibody herceptin. The single amino acid alteration H1047R within the kinase domain of PIK3CA is one of three hot spot mutations prevalent in BC.

Previously, we demonstrated that introduction of H1047R mutation in HER2-overexpressing MCF10A mammary epithelial cells enhances cellular transformation and decreases herceptin sensitivity by inducing secretion of endogenous ErbB ligand heregulin. However, genetic ablation of HER3, the major co-receptor for HER2 and the solitary receptor for heregulin, was insufficient for complete inhibition of cell growth, indicating the existence of additional mechanism/s responsible for the heightened aggressiveness and decreased drug sensitivity of HER2/H1047RPI3K cells. In the current study, we looked further into the molecular changes within these cells that might be responsible for these phenomena.

When compared with the HER2/WTP13K cells, the HER2/H1047RPI3K cells revealed a significant increase in CD44^{high}/CD24^{low/negative} populations, common markers of BC stem cells, as well as molecular and phenotypic changes associated with epithelial-to-mesenchymal transition. These observations are in agreement with previously published report on mouse model of HER2/H1047RPI3K BC. Further analyses demonstrated additional stem cell-associated characteristics in HER2/H1047RPI3K cells, such as expression of angiogenic and inflammatory cytokines, ability to induce chemotaxis and invasion, activation of TGFb and NFkB signaling pathways. Connectivity map (CMap) analysis of the gene expression signatures from HER2/H1047RPI3K cells revealed a negative association with those from BC cells treated with 17AAG, an inhibitor of the heat shock protein 90 (HSP90). In line with this, HER2/H1047RPI3K-expressing cells are found to be more sensitive to HSP90 inhibition compared to the pan-ErbB inhibitor lapatinib.

Cancer stem cells are implicated in drug resistance and tumor recurrence. Enrichment of cell population expressing high levels of stem cell markers and stem cell-related features could be one of major mechanisms by which BC cells co-expressing HER2 and H1047RPI3K adapt to anti-HER2 therapeutic agents. Acute dependence on molecular chaperone HSP90 provides a unique, yet practical opportunity to effectively inhibit tumors harboring both molecular alterations, since HSP90 inhibitors have already shown encouraging clinical activity in herceptin-resistant setting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-08-11

Title: UHRF1 promotes breast cancer progression via suppressing KLF17 expression

Gao S-P, Sun H-F, Li L-D and Jin W. Key Laboratory of Breast Cancer in Shanghai, Collaborative Innovation Center of Cancer Medicine, Fudan University Shanghai Cancer Center, Shanghai, China and Shanghai Medical College, Fudan University, Shanghai, China.

Body: Background: UHRF1, also termed as ICBP90 or Np95, is reported to be an epigenetic regulator of DNA methylation and histone modifications, which takes pivotal functions in cell differentiation and tumorigenesis. Here, we show that UHRF1 is markedly increased in breast cancer tissues and cell lines. And patients with high UHRF1 expression have a poor prognosis compare with those in low UHRF1 expression as showed by Kaplan Meier plotter analysis. However, the underlying mechanisms of UHRF1 in breast cancer remain largely unknown. Thus, the aim of this study is to validate potential mechanisms of UHRF1 in breast cancer cells.

Methods: The stable cell lines were constructed using interference and overexpression methods and the efficiency was confirmed by western blot. Then cell proliferation and migration were evaluated by CCK8 and transwell assay. The MDA-MB-231 pCDH and pCDH-UHRF1 cells were inoculate into mouse left flank to further testify UHRF1 function in vivo. Furthermore, microarray and ChIP-seq were implemented to detect potential mechanisms and the candidate genes were assessed by ChIP and qPCR. The BSP or MSP primers were designed by the methprimer database. Genomic DNA was prepared from breast cancer cells and then the genomic DNA was bisulfite modified using EZ DNA Methylation-Gold Kit. The methylation PCR products were cloned into pMD-18T and ten positive clones were sequenced. The data were analyzed using the QUMA analyzer software.

Results: We found that UHRF1 was indeed increased in breast cancer tissues and cells. And patients with high levels of UHRF1 were likely to have a shorter disease-free survival than patients with low levels. UHRF1 overexpression can promote cell proliferation and migration while UHRF1 downregulation have inverse functions. In vivo, UHRF1 also accelerate tumor growth on mice. Mechanistic studies revealed that 9 candidate genes were obtained by analysis from ChIP-seq and microarray databases. The further verification showed that Krüppel-like factor 17 (KLF17) is the most significantly upregulated one when UHRF1 gene was depleted, with rich CpG islands on its promoter region. We also observed that an inverse relationship between the expression of UHRF1 and KLF17 both in breast cancer cell lines and tissues. What's more, cells proliferation and migration which were inhibited by UHRF1-depletion can be rescued by KLF17 silence, suggesting UHRF1 promote breast cancer proliferation and migration through downregulating KLF17 expression. Moreover, overexpression of UHRF1 increases the methylation of CpG nucleotides and reduces the expression of KLF17 while depletion of UHRF1 decreases the methylation of CpG nucleotides with elevating expression of KLF17.

Conclusion: Our results demonstrate that increased UHRF1 can promote breast cancer cell proliferation and migration by epigenetic silencing of KLF17 expression through CpG islands methylation in its promoter region. These results provide insight into the breast cancer progression process and suggest that making changes in this mechanism represent new therapeutic approaches to block breast cancer development.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-08-12

Title: p53 status and 17q21.3 amplicon formation in HER2-positive breast cancer

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Body: Breast cancers that overexpress *HER2/neu* are associated with poor clinical outcome. Treatment of HER2-positive breast cancers with trastuzumab, a monoclonal antibody that antagonizes HER2 receptor signaling, increases patient overall and disease-free survival. While targeted therapy is reasonably effective, resistance to trastuzumab remains a problem, particularly in the case of metastatic disease. Tumor suppressor p53 is the most commonly mutated gene in human cancer and mutations that lead to the stabilization and accumulation of p53 in HER2-positive breast cancers are associated with worse clinical outcome. Another feature of HER2-enriched breast cancers are amplifications of the HER2 locus on chromosome 17q21.3.

Table 1

	Wildtype p53	Mutated p53
No amplification	12.8%	63.9%
17q21.3 amplification	13.1%	8.7%

Table 1: Among 58 cases, Her2-positive tumors with mutated p53 (n=37, 63.9%) occurred at a 4.9-fold higher frequency than amplification of the 17q21.3 gene set (n=8, 13.1%), with significant mutual exclusivity (p=0.005). The patient genomic data set was obtained from the Breast Invasive Carcinoma Study conducted by the TCGA Network (Nature, 2012) and visualized using cBioPortal (MSKCC). Copy alterations of genes were assessed using GISTIC2.0 (Broad Institute) via cBioPortal.

We studied the genomic profiles of 58 HER2-positive breast tumor samples using cBioPortal to determine p53 mutation status. 74.1% of samples expressed mutant p53 and a large fraction of mutations occurred in the key DNA binding domain. We assessed the amplification status of 24 genes within the chromosome 17q21.3 locus as an indicator of amplicon formation, and found that 21.8% of breast tumors demonstrated copy number amplification (Table 2). Mutant p53 tumors with no amplicon formation occurred 5 times more frequently than tumors with only 17q21.3 amplicon formation. These alterations tended to occur exclusive of one another (p=0.005, Table 1). Separately, using gene expression data from Kaplan-Meier Plotter, we observed that alterations in gene expression within the 17q21.3 amplicon can have differential effects on the survival of HER2-positive breast cancer patients (Table 2).

Table 2

17q21.3 Gene Set	Hazard Ratio	P-value
COL1A1	1.76	0.01
MBTD1	0.53	0.01
SPATA20	0.65	0.04
UBE2Z	0.68	0.07
EME1	0.65	0.08

Table 2: List of relevant genes within the chromosome 17q21.3 amplicon. Genes in this amplicon can influence both beneficial and hazardous survival outcomes in HER2-positive breast cancer patients (n=208). Hazard ratios (HR) were determined from gene expression data available through Kaplan-Meier Plotter (Gyorffy, 2010).

Our data shows that HER2-positive breast cancers can be divided into p53 mutant and non-mutant subsets with p53 mutations relatively exclusive to 17q21.3 gene amplification. However, p53 mutation status and 17q21.3 copy number have a variety of effects on patient outcome. We are interested in understanding the interaction between these two genetic alterations and whether subdividing HER2-positive breast cancer into these subtypes will improve our ability to provide effective therapy to patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-08-13

Title: The clinical importance of nuclear wild-type p53-induced phosphatase 1(Wip1) expression in breast cancer patients

Inoue Y, Yamashita N, Tokunaga E, Kitao H, Tanaka K, Saeki H, Oki E and Maehara Y. Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan and 2) National Hospital Organization Kyushu Cancer Center, Fukuoka, Japan.

Body: *Backgrounds;* The wild-type p53-induced phosphatase 1(Wip1) is a member of the serine/threonine protein phosphatases, and plays an important role in the nucleus as one of the key components in the DNA damage response network. Wip1 is encoded by the protein phosphatase magnesium dependent 1 delta (*PPM1D*), sited on locus 17q23. *PPM1D* gene amplification and/or Wip1 expression have been observed in numerous tumors, including breast cancer. *PPM1D* is referred to as oncogene, as Wip1 inhibits phosphorylation of p53 and work as a negative regulator in cell death. Inhibition of Wip1 may have an important therapeutic role in suppressing tumor growth and evolution.

Aims; We evaluated the expression of *Wip1* mRNA, Wip1 protein and *PPM1D* DNA copy number to clarify the relationship between Wip1 expression and the clinicopathological features and prognosis to determine the biological significance of Wip1.

Materials and Methods; Breast cancer cell lines (MCF7, T47D, MDA-MB231, HCC1937, HS578T, BT20 and SKBr3) were used for Wip1 expression analysis and copy number analysis. The specimens were obtained from Japanese breast cancer patients who underwent surgery without neoadjuvant chemotherapy or endocrine therapy in our department. *Wip1* mRNA expression was evaluated in 140 cases by quantitative RT-PCR and Wip1 protein expression was evaluated in 192 cases by immunohistochemistry (IHC). The *PPM1D* DNA copy number was analyzed by genomic PCR in 33 breast cancer cases and by SNP-CGH array (Illumina, Human Omni 2.5-8) in 12 cases. The effects on the cell growth of the Wip1 inhibitor (GSK2830371) were analyzed by the viability assay in MCF7.

Results; *Wip1* mRNA expression was significantly higher in MCF7, luminal type cell line. There was no significant correlation between *Wip1* mRNA expression and prognosis. In IHC, positive nuclear Wip1 protein expression was detected in 21 cases (10.9%). There was no significant correlation between *Wip1* mRNA expression and Wip1 protein expression. There was no significant association between the Wip1 protein expression levels and the clinicopathological factors and the prognosis. *PPM1D* DNA copy number significantly correlated with Wip1 protein expression ($p=0.0035$). Copy number gain at 17q23 was detected in 6 cases by SNP-CGH array, and all of these six cases showed positive nuclear Wip1 expression. *PPM1D* copy number gain was not observed in Wip1 negative cases. In the cell viability assay, the MCF7 cell growth was suppressed by Wip1 inhibitor administration.

Conclusions; Wip1 protein expression in nucleus is important as oncogene, and its expression may be regulated by *PPM1D* copy number gain. Wip1 is considered to be the new therapeutic target for breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-01

Title: Impact of MAF gene amplification on disease recurrence and effects of adjuvant zoledronic acid in early breast cancer

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Body: Background: Meta-analysis of clinical trials has shown that adjuvant bisphosphonates reduce bone metastases and improve survival in postmenopausal (PM) pts¹. However, we are unable to select pts most likely to benefit. To address this, the recently identified early breast cancer bone relapse biomarker, 16q23(MAF) gain (MAF+)², was tested retrospectively in the large prospectively randomized AZURE trial³ of standard adjuvant therapy +/- zoledronic acid (ZOL) to determine the prognostic value of MAF and its potential to predict the effects of ZOL on disease outcomes.

Materials and methods: All analyses were performed with ethics approval and consent. The biomarker analysis was completed on TMAs from primary tumors. Quadruplicate cores of breast tumor tissue were arrayed across replicate TMAs. MAF+ was detected using a validated (MAF/D16Z3) FISH test (Inbiomotion SL, Barcelona, Spain). A central laboratory (Targos, Kassel, Germany) validated the assay for analytic and diagnostic performance, established acceptance criteria, included appropriate quality controls for each assay, and performed the analyses in a blinded fashion. A copy number cut-off ≥ 2.5 was preset for MAF+. Invasive disease free (IDFS), overall (OS) survival and time to bone metastases multivariate analyses were performed in control and ZOL pts separately. Subsequently, interactions between MAF+ and effects of ZOL on disease outcomes by menopausal status were evaluated.

Results: 1769 of the 3360 AZURE pts donated primary tumor samples. Median follow-up was 84 months. 865 pts (49%) had 2 FISH evaluable cores and were included in the analysis of which 184 (21%) had MAF+ tumors. Tumors that were MAF+ were more likely to be of higher grade, ER-ve and Her2+.

In control pts, MAF was not prognostic for IDFS or OS although there were differences in IDFS by menopause (HR for MAF-/MAF+ in PM=0.47 [95%CI 0.25-0.88]; HR in non-PM=1.58 [0.82-3.03], test for interaction (TFI) by menopause P=0.007). In ZOL pts, MAF was prognostic for IDFS (HR=0.52 [0.36-0.75] and OS (HR=0.48 [0.31-0.75]). There were insufficient bone events (19 MAF+, 73 MAF-) in this sample set to reliably assess the impact of MAF+ on relapse in bone.

In pts with MAF- tumors, ZOL was associated with improved IDFS (HR=0.74 [0.56-0.98]) and OS (HR=0.78 [0.55-1.10]).

However, the effects of ZOL in MAF+ were profoundly influenced by menopausal status with possibly better outcomes in PM women (HR for IDFS=0.74 [0.35-1.58]) but clearly worse IDFS and OS outcomes in ZOL treated MAF+ pts who were non-PM (HR for IDFS 2.46 [1.23-4.92], TFI by treatment P=0.002 and HR for OS=2.27 [1.04-4.93], TFI by treatment P=0.032). The interactions between disease outcomes, ZOL use and menopause were driven largely by an association between MAF+ and an increased risk of extra-skeletal recurrence with the use of ZOL in women who were not PM.

Conclusions: Absence of MAF amplification is associated with improved disease outcomes with adjuvant ZOL. However, the use of adjuvant ZOL in women with MAF+ tumors who are not PM at the start of treatment is associated with extraskelatal spread and worse DFS and OS.

¹EBCTCG Lancet 2015;386:1353–1361; ²Pavlovic M et al JNCI 2015;107(12):djv256; ³Coleman RE et al Lancet Oncol 2014;15:997-1006.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-02

Title: Homologous repair deficiency (HRD) as measure to predict the effect of carboplatin on survival in the neoadjuvant phase II trial GeparSixto in triple-negative early breast cancer

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Body: Introduction

Addition of carboplatin to anthracycline/taxane-based neoadjuvant chemotherapy has shown to improve pathological complete response (pCR; ypT0 ypN0) rates in patients with triple-negative breast cancer (TNBC) in two large phase II studies (GeparSixto: von Minckwitz et al, Lancet Oncol 2014, CALGB 40603: Sikov WM, J Clin Oncol 2015). Participants of the GeparSixto study showed an improvement of pCR rate from 36.9 to 53.2% ($p=0.005$) and DFS by absolute 9% (HR 0.56 95% CI 0.33-0.96] $p=0.035$) with the addition of carboplatin in the TNBC subgroup. No effect was observed in the HER2-positive subgroup. We here report results on homologous repair deficiency (HRD) status in relation to pCR and DFS in the TNBC subgroup.

Patients and Methods

In the GeparSixto trial (NCT01426880), patients were treated for 18 weeks with paclitaxel 80mg/m² q1w and non-pegylated-liposomal doxorubicin (NPLD) 20mg/m² q1w. Patients with TNBC (N=315) received concurrently bevacizumab 15mg/kg i.v. q2w until surgery. All patients were randomized 1:1 to receive concurrently carboplatin AUC 1.5-2 q1w vs no carboplatin. Carboplatin dose was reduced from AUC 2.0 to 1.5 by an amendment after 330 patients. Primary objective is pCR rate (ypT0 ypN0). Event free survival (EFS), and overall survival (OS) were secondary objectives. HR Deficiency status was assessed on FFPE material from pretherapeutic core biopsies. HR Deficiency was defined as either HRD score high or a *BRCA* mutation.

Results

HRD status was measurable in 193 of 315 TNBC patients. 101 patients of them were randomly assigned to receive carboplatin and 92 to no additional carboplatin. After median follow-up of 34.3 months 43 event free survival (EFS) events have been reported.

HR deficiency was detected in 136 (70.5%) tumors of which 79 (58.1%) showed high HRD score with intact *tBRCA*. HR deficiency independently predicted pCR (ypT0 is ypN0) (odds ratio (OR) 2.506, CI 1.243-5.051, $p=0.009$). Adding carboplatin to PM significantly increased the pCR rate from 36.6% to 63.2% in HR deficient tumors with intact *tBRCA* ($p=0.018$), only marginally from 61.9% to 72.7% in *BRCA* mutated tumors ($p=0.406$), and moderately from 20.0% to 40.7% in HR non-deficient tumors ($p=0.086$). In general, patients with HRD deficient tumors had a better EFS than non HRD deficient ones (HR 1.805 (0.985-3.309); $p=0.0526$). Patients with high HRD score had an insignificant trend towards an improved EFS compared to those with low HRD score (HR 1.546 (0.764-3.127) $p=0.2223$). HRD deficiency did not predict carboplatin effect in patients without *BRCA* mutation (HR 0.8617). In multivariable analysis, only therapy, clinical nodal status before treatment, and lymphocyte predominant breast cancer were significant prognostic on EFS.

Conclusion

Within the GeparSixto study HR deficiency (either HRD score high or *BRCA* mutation) was associated with a higher pCR in general and an improved EFS. The effect of carboplatin could not be predicted by HR deficiency in this relatively small study. However, the results will help to understand the role of HR deficiency and the value of the HRD score in TNBC especially in patients without *BRCA* mutation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-03

Title: Relative radioresistance in triple negative tumors in the SweBCG91-RT randomized clinical trial

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Body: Introduction: Breast-conserving surgery (BCS) with adjuvant whole breast radiation therapy (WBRT) is the standard treatment for a majority of early breast cancer patients. No predictive biomarkers for RT are in use and most patients are cured by surgery alone, and are thus over-treated. Further, some patients suffer a relapse despite WBRT, and may have benefited from mastectomy or more aggressive postoperative treatment. Gene expression tests can be used to predict risk of distant recurrence and effect of adjuvant systemic therapy, and can reveal the intrinsic subtype of the tumor. A surrogate method of determining intrinsic subtype based on high quality centralized immunohistochemistry (IHC) has been proposed with criteria set up by the St Gallen consensus conference 2013. The intrinsic subtypes provide prognostic information and are treatment predictive for chemotherapy, but the predictive potential for WBRT has not been conclusively determined.

Aim: To evaluate the effect of WBRT on ipsilateral breast tumor recurrence (IBTR), in patients with tumors of different intrinsic subtypes.

Methods: Tumor tissue from FFPE blocks were collected from 1003 breast cancer patients with node negative, stage I-II disease, randomized to BCS with or without WBRT, in the randomized SweBCG RT-91 trial between 1991-1997. Systemic adjuvant treatment was administered according to regional guidelines, but was sparsely used. Median follow-up was 15.2 years. Tissue microarrays were constructed and stained for estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2) and Ki-67. Centralized evaluation was performed by two pathologists subspecialized in breast pathology. Endpoint IBTR within 10 years was considered with a cumulative incidence and competing risks approach. P-values were calculated with the cause-specific logrank test and hazard ratios (HR) with cause specific Cox regression. Multivariate models, with or without an interaction term between subtype and WBRT, were compared to formally test if the effect of RT differs between subtypes.

Results: We were able to stain and score 963 out of 1003 tumors. These were classified as Luminal A-like (n=537), Luminal B-like (Her2-negative, n=261), triple negative (n=95) and HER2-positive (any ER status, n=70). WBRT reduced the frequency of IBTR for Luminal A-like tumors (19% vs 9%, HR 0.45 (0.28-0.73), p<0.001), Luminal B-like tumors (23% vs 6%, HR 0.23 (0.11-0.51), p<0.001) and non-significantly for HER2-positive tumors (24% vs 16%, HR 0.59 (0.20-1.75), p=0.33), but not for triple negative tumors (18% vs 16%, HR 0.86 (0.32-2.31), p=0.76). However, the overall difference in WBRT effect between subtypes was not formally statistically validated (p=0.20).

Conclusions: We found that WBRT reduced IBTRs among the Luminal A-like and B-like subgroups, and to a lesser extent in the HER2-positive subgroup, but not in the triple negative subgroup. Thus, intrinsic subtyping by IHC may give important information on how tumors respond to adjuvant WBRT, and patients with triple negative tumors may need more aggressive local treatment. Additional studies are required and it remains to study the effect on breast cancer specific survival.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-04

Title: Proliferation and p21 refine risk of relapse in residual disease after HER2-directed therapies

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Body: Background: Patients (pts) with residual disease (RD) after neoadjuvant therapy are at higher risk of relapse. We investigated whether biomarkers assessed at surgery in patients pts with RD in the NeoSphere study were informative for risk of distant event free survival (DEFS)

Methods: In NeoSphere 417 HER2+ pts were randomized to neoadjuvant TD, TPD, TP or PD (T=trastuzumab, P=pertuzumab, D=docetaxel), and received FAC/FEC and trastuzumab after surgery. 296 pts had RD. Affymetrix derived gene expression profiles (GEPs) were available at surgery for 201 pts (67.9%). 176 pts (60.1%) had paired samples before and after treatment with available GEPs. We investigated the prognostic value of proliferation evaluated by Mitosis Kinase Score (MKS) (Bianchini G Cancer Res 2010), and performed a gene discovery for association between gene expression at surgery and DEFS.

Results: MKS as continuous marker was associated with significantly higher risk of relapse when assessed at surgery (HR 1.80 [1.23-2.65]; $p=0.002$), but not before treatment (HR 1.50 [0.80-2.78]; $p=0.20$). In paired samples, there was an average decrease ($p=9.2E-11$) of MKS after treatment, which was prominent in ER+ and chemotherapy-containing arms. In ER- and TP arm there were cases of increase and of decrease of MKS. In ER+ the 5 years DEFS was 94.3% in the Low/Int MKS tertiles group (pooled) vs 70.5% in the High MKS tertile group (HR 5.41 [1.87-15.6]; $p=0.002$). In ER-, the 5 years DEFS was 85.0% in the Low/Int vs 64.1% in the High group (HR 2.89 [1.08-7.76]; $p=0.035$). Notably, MKS at surgery after the two monoclonal alone was also prognostic.

In the gene discovery approach only the expression of CDKN1A (p21) at surgery was associated with DEFS after correction for false discovery rate (FDR=0.01). Pre-treatment p21 was not associated with DEFS. Paired comparison showed significant upregulation of p21 in all patients, treatment arms and ER groups. The Int/High p21 tertiles group (pooled) had lower risk of recurrence than the low tertile in ER+ (HR 4.31 [1.60-11.6]; $p=0.004$) and in ER- (HR 5.81 [1.87-18.1]; $p=0.002$) groups. p21 in TP arm was also prognostic. MKS and p21 expression provided independent prognostic information and remained significant after correction for clinico-pathological variables (nodes and T stage) and tumor-infiltrating lymphocytes. Combining the two markers, there was a group at very low risk (Low/Int MKS and Int/High p21) and one at high risk (High MKS and Low p21). The other tertiles combinations had intermediate risk. In ER+, the 5 yrs DEFS was 94.9% in the low risk group and 52.9% in the high risk ($p=1.9E-05$). In ER-, the 5 yrs DEFS was 96.5% in the low and 45.5% in the high risk group ($p=0.001$). The markers' combination was also prognostic in the two monoclonal only arm.

Conclusions: Proliferation (MKS) and p21 expression are modulated by trastuzumab and/or pertuzumab regimens. Tumors with high MKS and low p21 in RD after neoadjuvant therapy defined a group at very high risk of relapse. Tumors with low/int proliferation and int/high p21 had low risk of recurrence similar to that of patients achieving pCR. Whether the pharmacodynamic modulation of p21 could be used as surrogate marker of long term benefit in patients with RD deserves additional investigation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-05

Title: The role of immune and apoptosis markers for prediction of pCR in the WSG-ADAPT HER2+/HR+ phase II trial evaluating 12-weeks of neoadjuvant TDM1 ± endocrine therapy (ET) versus T + ET in HER2-positive hormone-receptor-positive early breast cancer (EBC)

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Body: Background: Immune and apoptosis biomarkers are potential prognostic/predictive markers in HER2+ EBC. High PD-L1 expression was shown to be predictive for lower pCR after chemotherapy+trastuzumab+/-pertuzumab, particularly in HER2+, ER- disease. Yet, HER2+ EBC co-expressing hormone receptors is a distinct entity.

The ADAPT HER2+/HR+ phase II trial (n=376) compared 12 weeks of neoadjuvant T-DM1 + ET vs. trastuzumab (T)+ET and demonstrated pCR rates of about 41% in both (well tolerated) T-DM1 arms.

Methods: In order to identify potential early predictors for pCR (i.e. no invasive tumor in breast and lymph nodes), immune markers (PDL1 on infiltrating immune cells (IIC) and on tumor cells (TC); CD8 in invasive margin and in tumor center) and apoptosis markers (bcl-2; mcl-2) were determined by immunohistochemistry (IHC; H-scores) in core biopsy sections obtained at primary diagnosis and at cycle 2. For multivariate logistic regression, each biomarker (separately), clinical factors (Ki-67, cT, cN) and therapy were entered. All analyses were exploratory.

Results: Biomarkers were available in up to 326 patients (pts) at baseline and up to 170 pts at 3 weeks (due to low tumor content in 2nd core biopsy).

Baseline IIC-PDL1 was associated with pCR in the T-DM1 arm (OR 2.89; 95%CI: 1.11-7.51); IIC-PDL1 at cycle 2 was not associated with pCR.

PD-L1 expression in TC was rare (2%); cycle-2 TC-PD-L1 was associated with pCR in all pts and in the pooled TDM-1 arms. High baseline CD8 in tumor center was associated with pCR in the whole cohort (OR 2.4; CI: 1.04 – 5.5) and in the T+ET arm (OR=10.1; CI: 1.12 - 91.6) and at cycle 2 in all pts (OR=9.52; CI: 2.17 – 41), in pooled TDM-1 arms (OR=15.7; CI: 2.49 – 99), and in TDM-1+ET (OR=25.05; CI: 2.12 – 295). Increases in this marker also predicted pCR in all pts, pooled TDM-1, and in TDM-1+ET. Association of cycle-2 CD8 in tumor center with pCR persisted in multivariate models.

Lower baseline CD8 in invasive margin was associated with pCR in the T-DM1 arm (OR=0.09; CI: 0.01-0.69), but at cycle 2 in all pts (OR=18.1; CI: 1.60 – 204) and in pooled TDM-1 arms (OR=23.5; CI: 1.1 - 500). This positive impact persisted in multivariate models.

Bcl-2 expression at baseline was associated with non-pCR in all pts (OR=0.28, CI: 0.12 - 0.66), in the pooled T-DM1 arms (OR=0.216, CI: 0.08 - 0.61), and particularly in the T-DM1+ET arm (OR=0.14; CI: 0.03 - 0.71). This association persisted in multivariate analysis. At cycle 2, lower bcl-2 had OR=0.16 (CI: 0.03 - 0.96) in the pooled T-DM1 arms. No association with efficacy was seen for mcl-1.

Conclusions: The WSG-ADAPT HER2+/HR+ phase II trial is the first international trial to focus on HER2+/HR+ EBC alone and the first to show substantial pCR rates of > 40% after only 12 weeks of T-DM1 -- without standard chemotherapy. Expression of bcl-2 may affect resistance to T-DM1. High immune activity at baseline and/or cycle 2 seems to be associated with

pCR. The association of CD8 expression and its changes with therapy efficacy is complex and could depend on ET. Further biomarker analyses are ongoing and will be presented at the meeting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-06

Title: Prognostic and predictive relevance of cell cycle progression (CCP) score in ductal carcinoma *in situ*: Results from the UK/ANZ DCIS trial

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Body: Background: The prognostic abilities of most gene expression signatures in breast cancer are often due to detection of proliferative activity measured from expression of genes regulated as a function of cell cycle progression. Cell Cycle Progression (CCP) score is an important prognostic factor in prostate cancer, and has shown promising results for renal and lung cancer; its role in ductal carcinoma *in situ* (DCIS) has not been explored. We investigated the prognostic and predictive relevance of CCP Score in DCIS using material from UK/ANZ DCIS trial.

Methods: Formalin-fixed paraffin embedded tissues were collected from patients enrolled in the UK/ANZ DCIS trial, a randomised 2X2 factorial design trial investigating role of tamoxifen, radiotherapy (RT) or both as adjuvant treatment in DCIS. mRNA expression of 25 S- and M-phase CCP genes was evaluated by reverse transcription followed by PCR on customized Taqman low-density arrays. CCP score is an un-weighted average of the expression values of CCP genes after normalisation with 14 housekeeping genes. CCP score was analysed as a continuous variable and also as an ordinal variable using tertile-based cut-offs. Exploratory analyses with subgroups defined by HER2 status by immunohistochemistry were performed.

Results: CCP scores were evaluable in 521 (134 recurrence events) of 704 available samples (DCIS absent or insufficient RNA in 51, assay failure in 132). Increase in CCP score (median 1.15; IQR 0.71-1.74) was associated with increased risk of ipsilateral breast event (IBE) [Hazard ratio (HR) = 1.28; 95% Confidence Interval (95%CI) 1.08-1.51; p = 0.0049]. CCP score however was not an independent predictor in multivariate analyses [HR = 1.16; 95%CI 0.95-1.42; p = 0.14].

CCP scores were categorised as CCP low (<0.87), CCP intermediate (>= 0.87 to < 1.52) and CCP high (>= 1.52) by tertiles.

The benefit of RT in reducing IBE was significant when CCP score was low [HR = 0.35; 95%CI 0.14-0.87; p = 0.024] or intermediate [HR = 0.23; 95%CI 0.09-0.59; p = 0.0023], however, those with high CCP score did not derive significant RT benefit [HR = 0.59; 95%CI 0.31-1.13; p = 0.11].

In exploratory subgroup analyses, HER2 negative DCIS with high CCP score (20.9% of all DCIS cases) did not derive RT benefit and the largest RT benefit was seen for DCIS that expressed HER2 and did not have a high CCP score (23.2% of all DCIS cases).

Benefit of RT and 10-year IBE rates by CCP score (categorised) and HER2 status subgroups.

Subgroup	n	events	HR (95%CI)	p	10-year IBE rates (%) - No RT	10-year IBE rates (%) - RT
CCP-high & HER2 neg	106	22	0.83 (0.35-1.97)	0.67	22.5 (14.0-35.0)	20.0 (10.5-36.0)
CCP-high & HER2 pos	67	21	0.43 (0.16-1.17)	0.098	40.6 (27.1-57.6)	20.4 (8.9-42.9)
CCP-non-High & HER2 neg	217	30	0.43 (0.18-0.99)	0.048	16.2 (10.7-24.0)	8.1 (4.0-16.3)
CCP- non-High & HER2 pos	118	33	0.14 (0.04-0.46)	0.0012	39.5 (29.3-51.6)	7.1 (2.3-20.4)

CCP-non-High = low or intermediate CCP score

Conclusions: CCP score is not independently associated with the risk of IBE but appears to be a predictor of RT benefit.

Exploratory analyses suggest that combined with HER2 status, it may help in identifying a large DCIS subgroup where RT is

highly indicated and another large subgroup where mastectomy may be merited.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-08

Title: Predictive effect of cytotoxic tumor infiltrating lymphocytes in HER2-positive metastatic breast cancer: A correlative study with CCTG MA.31

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Body: Background and Objectives: The presence of tumor infiltrating lymphocytes (TILs), particularly CD8+ cytotoxic T-cells, has been associated with improved prognosis in patients with HER2+ breast cancer. Increasing levels of TILs also appear to predict response to adjuvant trastuzumab in early breast cancer, although they did not predict benefit of combined trastuzumab-lapatinib neoadjuvant dual therapy over monotherapy in NeoALLTO. CCTG MA.31 randomized 652 women with HER2+ metastatic breast cancer to treatment with trastuzumab (T) vs. lapatinib (L), in combination with taxane (Tax) chemotherapy for 24 weeks, followed by the same HER2-targeted monotherapy. Final results from MA.31 found trastuzumab was superior to lapatinib for the primary endpoint of progression free survival (PFS): the hazard ratio (HR) for lapatinib to trastuzumab was 1.37 (95% CI, 1.13-1.65). Although both agents block HER2 signaling, trastuzumab has additional mechanisms of action via the immune system. We hypothesized that TIL levels may predict response to HER2-targeted therapy (trastuzumab vs. lapatinib).

Methods: MA.31 included HER2+ metastatic breast cancer patients, median age 55 years, and median follow-up 21.5 months. Overall TILs were counted per published guidelines on the original H&E stained sections used for pathology review at study entry. Immunohistochemistry (IHC) was performed on unstained sections from tissue microarrays or individual formalin-fixed paraffin-embedded blocks to test expression of lymphocyte biomarkers CD8, FOXP3, CD56 and PD-1 on stromal and intra-tumoral TILs (sTILs, iTILs). Statistical analysis was conducted by CCTG for a total of 9 prespecified biomarker tests. Associations of TILs with PFS were evaluated by univariate stratified log-rank test with graphical Kaplan-Meier curves, and by stratified multivariate Cox proportional hazards regression analysis. Predictive effect was examined with a test of interaction between treatment allocation and biomarker classification (high vs. low, using pre-established cutpoints).

Results: Of the 652 cases, 614 had slides for overall TIL assessment and 427 for IHC biomarker assessments. In this correlative study set, superiority of trastuzumab over lapatinib for PFS was confirmed in multivariate analysis (LTax/T vs. TTax/L: HR = 2.55, 95% CI = 1.43-4.55, p = 0.001). TIL counts by H&E were neither prognostic nor predictive in this set of metastatic HER2+ breast cancers. Lymphocyte IHC markers were not prognostic. However, prespecified stratified univariate analysis detected a significantly higher risk for lapatinib over trastuzumab (HR = 2.94, 95% CI = 1.40-6.17, p = 0.003) in patients with low CD8+ sTIL (< 3) than was observed among those with high CD8+ sTIL (HR = 1.36, 95% CI = 1.05-1.75, p = 0.019). This differential effect was confirmed in multivariate analysis (interaction test p = 0.042). The other tested biomarkers did not demonstrate significant predictive effects.

Conclusions: In this correlative study of metastatic HER2+ breast cancer, a low level of pre-existing stromal cytotoxic T cell infiltration predicts women who benefit most from trastuzumab over lapatinib. Overall TIL counts were neither prognostic nor predictive.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-09

Title: Efficacy and gene expression results from SOLTI1007 NEOERIBULIN phase II clinical trial in HER2-negative early breast cancer

Prat A, Ortega V, Villagrasa P, Paré L, Galván P, Oliveira M, Nucíforo P, Lluch A, Morales S, Amillano K, Lopez R, Gonzalez R, Manso L, Martinez J, Llombart A, De la Peña L, Di Cosimo S, Rubio IT T, Harbeck N, Baselga J and Cortés J. Hospital Clinic i Provincial, Barcelona, Spain; Translational Genomics Group, Vall d'Hebron Institute of Oncology, Barcelona; Hospital Universitari Vall d' Hebron, Barcelona; SOLTI Breast Cancer Research Group, Barcelona; Translational Genomics and Targeted Therapeutics in Solid Tumors. Institut de Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona; Hospital Clinico Universitario, Valencia; Hospital Universitari Arnau de Vilanova, Lleida; Hospital Sant Joan de Reus, Tarragona; Complejo Universitario de Santiago de Compostela, A Coruña; Hospital Universitario Virgen del Rocio, Sevilla; Hospital Universitario 12 de Octubre, Madrid, Spain; Hospital Universitario Virgen de la Arrixaca, Murcia, Spain; Hospital Universitario Arnau de Vilanova, Valencia, Spain; Instituto Nazionale dei Tumori, Milan, Italy; Breast Center of the University of Munich, Munich; Memorial Sloan Kettering, New York and Hospital Universitario Ramon y Cajal, Madrid.

Body: Background: Eribulin is the only cancer agent that has demonstrated a significant prolongation in overall survival on previously treated breast cancer patients. To date, no biomarker exists to prospectively select patients who will derive the maximum benefit from this chemotherapeutic. In the SOLTI1007-NeoEribulin study, we explored, in a prospective clinical trial, the efficacy and the association of pre-treatment expression of RNA in patients with HER2-negative breast cancer treated with neoadjuvant eribulin.

Methods: SOLTI1007 is a phase II, open-label, single-arm, exploratory pharmacogenomic study of single agent eribulin as neoadjuvant treatment for stage I-II HER2-negative breast cancer (planned n=100 hormonal receptor-positive [HR+] and n=100 HR-negative). Patients received 1.4 mg/m² of eribulin intravenously on Days 1 and 8 every 21-day cycle, for 4 cycles. Baseline and post-treatment (surgical) formalin-fixed, paraffin-embedded tissue samples were collected and gene expression profiled. PAM50 intrinsic subtype and the Risk of Relapse based on subtype and proliferation (ROR-P) were evaluated in each time-point. The association of each PAM50 signature and pathological complete response in the breast (pCR_B) was evaluated using univariate logistic regression models.

Results: Between September 2012 and October 2015, one hundred and seventy-four patients (TNBC n=73 and HR+ n=101) were recruited. Mean age (55.5), stage II (90%), negative axilla (78% and 67%), grade 3 (62% and 26%), mean tumor size (3 cm and 3.6 cm) and mean Ki-67 (61% and 31%). Completion of 4 cycles of eribulin was achieved by 85% of the patients. Grade 3-4 toxicities were observed in 19.54%, mostly due to neutropenia (5.1%) and alopecia (4.02%). The overall pCR_B was 5.4%. No significant differences were observed between HR+ and TNBC disease. Distribution of the PAM50 intrinsic subtypes was as follows: Luminal A (n=43, 27.7%), Luminal B (n=42, 27.1%), Basal-like (n=63, 40.6%) and HER2-enriched (n=7, 4.5%). pCR_B rates by subtype were the following: HER2-enriched (28.6%, 2/7), Luminal B (7.1%, 3/42), Basal-like (4.8%, 3/63), Luminal A (2.3%, 1/43). pCR_B rates significantly (p=0.047) differed when HER2-enriched was compared to the other subtypes (odds ratio = 8.06, 95% CI 1.32-49.1). pCR_B rate differed significantly by ROR-P (p=0.006): ROR-P high (17.1%, 6/35), ROR-P med (2.7%, 2/75), ROR-P low (2.2%, 1/45). Ki67 % by IHC did not predict pCR_B (p=0.918). Subtype change at surgery occurred in 60% (3/5) HER2-enriched, 44.1% (15/34) of Luminal Bs, 10.3% (4/39) of Luminal A and 5.4% (2/37) of Basal-like tumors. 100% of subtype changes in Luminal B disease were to Luminal A.

Conclusions: From a response and biological perspective, patients with HER2-enriched and Luminal B disease may benefit the most from eribulin therapy. Mechanistically, our gene expression data further supports previous preclinical evidence suggesting that eribulin triggers a phenotypic conversion.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-10

Title: Pathological complete response to neoadjuvant trastuzumab is dependent on HER2/CEP17 ratio in HER2-amplified early breast cancer

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Body: Purpose To evaluate whether pathological complete response to neoadjuvant trastuzumab is dependent on the level of HER2 amplification.

Patients and Methods 114 women with HER2-overexpressing early breast cancer who had received neo-adjuvant trastuzumab in the prospective ABCSG-24 and ABCSG-32 trials, and for whom the HER2/CEP17 ratio was available, were included in this analysis. The ratio was correlated with tumor response as measured by the three most commonly used definitions of pathological complete response: ypT0 ypN0, ypT0/is ypN0, and ypT0/is.

Results In trastuzumab-treated patients, ypT0 pN0 was achieved in 69.0% of patients with a HER2/CEP17 ratio of >6, but only in 30.4% of tumors with a ratio of ≤6 (p=0.001, Chi Square test). When pCR was defined by ypT0/is pN0 or by ypTis, 75.9% and 82.8% of tumors with a high ratio had a complete remission, while only 39.1%, and 38.3% with a low ratio achieved a pCR (p=0.002 and p<0.001, respectively). Logistic regression revealed that tumors with a higher HER2/CEP17 ratio had a significantly higher probability to achieve ypT0 ypN0 (OR: 5.08, 95% CI 1.86-13.90; p=0.002) than tumors with a low ratio, while none of the other clinicopathological parameters was predictive of pCR. The association between high HER2 amplification and pCR was almost exclusively confined to HR positive tumors (62.5% vs. 24.0%, 75.0% vs. 28.0%, and 87.5% vs. 28.0%, for ypT0 ypN0, ypT0/is ypN0, and ypT0/is; p=0.014, p=0.005, and p<0.001), and was largely absent in HR negative tumors.

Conclusion A HER2/CEP17 ratio of >6 in the pre-therapeutic tumor biopsy is associated with a significantly higher pCR rate particularly in HER2 / HR co-positive tumors, and can be used to predict outcome before neoadjuvant trastuzumab is initiated.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-11

Title: Outcome after neoadjuvant chemotherapy in progesterone receptor negative breast cancer patients – A pooled analysis of individual patient data from ten prospectively randomized controlled neoadjuvant trials

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Body: Background

The estrogen receptor (ER) as a nuclear transcription factor alters the transcription of estrogen sensitive genes to which the progesterone receptor gene belongs. The ER has also been described to exert non genomic effects by interacting with several cell signalling pathways that do not initially involve increases in gene transcription. These different patterns of action of the ER lead to the assumption that in tumors that utilize the non-genomic ER activity in order to stimulate tumorigenesis and proliferation progesterone receptor (PgR) expression would be decreased or absent. Therefore lack of PgR expression could be a surrogate marker of altered growth factor signalling. The aim of this study was to investigate if PgR expression may act as a predictive factor for response to neoadjuvant chemotherapy and long-term outcome in breast cancer patients.

Methods

5613 patients with primary breast cancer, follow-up, positive ER expression; HER2+ and HER2- from overall 10 (n=9785) German neoadjuvant trials receiving an anthracycline and taxane based chemotherapy were included. The pathologic complete response (pCR)(ypT0, ypN0), long term survival data (disease free survival (DFS), distant disease free survival (DDFS), overall survival (OS) and local recurrence free survival (LRFS)) and early relapse, defined as DFS <37 months, were compared according to their PgR expression, overall and in subgroups defined by HER2.

Results

Tumors lacking PgR expression (1172 patients) were more often of grade 3 (38.4% v 26.3%; p<0.001), tended to have an advanced clinical nodal involvement (6.8% v 4.7%; p=0.004) and were more likely to demonstrate HER2 positivity (36.2% v 22.3%; p<0.001).

pCR rates were significantly higher in PgR negative patients in the entire cohort (13.8% v 7.5%; p<0.001) as well as in the HER2 negative subgroup (11.2% v 5.8%; p<0.001) whereas there was no significant difference in the HER2 positive (22.1% v 18%; p=0.117). After adjusting for known predictive factors in the multivariable logistic regression analysis PgR negativity was an independent predictive factor for pCR overall (OR 1.76; p<0.001) and in the HER2 negative patients (OR 1.99; p<0.001).

PgR negativity was also significantly associated with an early relapse overall (32.8% v 25.7%; p<0.001) and in the subgroups defined by HER2 (HER2- 32.2% v 24.9%; p<0.001 and HER2+ 39.9%v 30.5%; p=0.002).

Patients with PgR negative disease had a significantly worse DFS, OS, DDFS and LRFS (p<0.001, respectively). Multivariable Cox regression analysis revealed that PgR was an independent prognostic factor. This was also observed in the HER2+ and-subgroups. Interestingly, in the PgR negative tumors HER2 status did not influence long-term outcome.

Conclusion

This analysis demonstrates that ER positive and PgR negative tumors represent a specific subset in primary breast cancer patients associated with higher response but also worse long term outcome after neoadjuvant chemotherapy. Interestingly, PgR negativity served as an independent predictive factor for achieving a pCR after neoadjuvant chemotherapy and therefore its status should be considered when deciding on systemic treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-12

Title: Dose dense adjuvant chemotherapy in patients with early breast cancer: Differential treatment effects according to composite index of benefit

Puglisi F, Ceppi M, Cognetti F, De Placido S, Bruzzi P, De Laurentiis M, Bisagni G, Cavazzini G, Durando A, Turletti A, Valle E, Montemurro F, Barni S, Ardizzoni A, Gamucci T, Colantuoni G and Del Mastro L. University Hospital of Udine, Udine, Italy; Gruppo Italiano Mammella, Italy and IRCCS AOU San Martino-IST, Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy.

Body: Background In patients with node-positive early breast cancer (EBC), dose-dense adjuvant chemotherapy improves disease-free survival (DFS) compared with standard interval chemotherapy. The GIM2 trial supports the value of dose-dense chemotherapy and suggests that the benefit is present in patients with hormone receptor-negative or hormone receptor-positive tumours (Del Mastro et al. Lancet 2015). In order to individualize decision making, there is a need to examine the absolute treatment effects of dose dense chemotherapy according to patient and tumor characteristics.

Patients and Methods The randomized phase III GIM2 trial enrolled 2091 patients. The primary endpoint was DFS. A continuous, composite measure of treatment benefit for each patient was determined from a Cox model incorporating potential predictive factors (age: 25-40/41-55/56-71; histological grade: 1+2/3; hormonal receptor status: positive/negative). Subpopulation treatment effect pattern plot methodology was used to reveal differential treatment effects on DFS according to composite index. The study focused on patients with HER2-negative disease (N=1287).

Results On average, the magnitude of benefit with dose dense chemotherapy versus standard chemotherapy ranged widely across different subpopulations, as quantified by the composite measure of relevant variables. The highest benefit was observed in patients with high grade, hormone receptor-negative disease (hazard ratio for DFS 0.44, 95% CI 0.23-0.83). Of note, a relevant benefit was observed also in patients with high grade, hormone receptor-positive disease (hazard ratio for DFS 0.74, 95% CI 0.50-1.09).

Conclusion The absolute improvement in DFS with dose dense adjuvant chemotherapy is substantial in some patients with node-positive HER2-negative breast cancer, particularly those regarded as having high index risk (hormone receptor-negative, high grade disease). Interestingly, a significant effect of dose dense chemotherapy was observed also in patients with hormone receptor-positive, high grade disease.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-13

Title: A RB-1 loss-of-function gene-signature (RBSig) predicts resistance to neoadjuvant chemotherapy in HER2+/ER+ breast cancer patients

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Body: Background: HER2+ breast cancers (BC) are clinically and biologically heterogeneous, with approximately half being ER+. Compared to other BC subtypes, HER2+ ER+ tumors display among the lowest rates of pathological complete response (pCR) following neoadjuvant chemotherapy (NACT) +/- anti-HER2 agents (anti-HER2). Yet in spite of this, HER2+/ER+ patients (pts) are typically treated with NACT plus anti-HER2, with the subsequent related toxicity. Currently there is a lack of predictive biomarkers that identify which subgroups of pts will not respond to such therapy. Inactivation of the Retinoblastoma (Rb) signalling pathway is a frequent event in BC. Previously developed gene-signatures of Rb loss-of-function have shown strong prognostic value and prediction of response to NACT. However, none has been extensively studied in the context of HER2+/ER+ BC. We have recently developed a gene-signature of RB-1 loss-of-function (RBSig) that is prognostic in luminal A-like and luminal B-like BC. Here we report the results of a retrospective *in-silico* study aimed to determine whether low expression of the RBSig in HER2+/ER+ BC correlates with a low pCR rate following NACT +/- anti-HER2.

Methods: We performed a PubMed search for clinical trials of NACT +/- anti-HER2 (trastuzumab, lapatinib, or both) in HER2+ BC pts, and selected studies which had available gene expression data, hormone receptors status and pCR information. *In-silico* analyses of correlation between RBSig expression and pCR were performed using receiver-operating characteristic (ROC) curves and Fisher exact test to assess the prediction performance of the signature score. The threshold RBSig score was set at the 50th percentile of the score distribution.

Results: Out of 16 identified studies, 10 fulfilled the inclusion criteria and were included in the analysis (514 pts). Overall, of the 211 HER2+/ER+ BC pts, 49 achieved pCR (23%); the pCR rate following NACT +/- anti-HER2 of pts with RBSig low expression was significantly lower compared to pts with RBSig high expression (16% vs 30%, respectively; Fisher exact test $p=0.0098$). The area under the ROC curve (AUC) was 0.62 (95% confidence interval (CI) 0.54-0.7, $p=0.005$). Results were similar for pts receiving NACT alone (94 pts; pCR rate 13% vs 28% in RBSig low vs RBSig high, respectively; Fisher exact test $p=0.06$; AUC 0.62, 95% CI 0.5-0.74, $p=0.043$) or combined with anti-HER2 (117 pts; pCR rate 18% vs 33% in RBSig low vs RBSig high, respectively; Fisher exact test $p=0.049$; AUC 0.61, 95% CI 0.5-0.72, $p=0.041$). In 303 HER2+/ ER- pts treated with NACT +/- anti-HER2, the pCR rate was 42%. No correlation was found between RBSig expression score and pCR rate in this group (pCR rate 42% vs 43% in RBSig low vs RBSig high, respectively; Fisher exact test $p=0.53$; AUC 0.5, 95% CI 0.43-0.56, $p=0.973$).

Conclusions: RBSig identifies a subset of HER2+/ER+ pts with a low pCR rate following NACT +/- anti-HER2. We hypothesize that this signature has the potential to identify pts for whom chemotherapy could be avoided in favour of combinations of endocrine therapy and target therapies. Further refinement and validation in an independent dataset is warranted.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-14

Title: Breast carcinoma with 21-gene recurrence score lower than 18: *Rate of distant metastases in a large series with clinical follow-up*

Wen HY Y, Krystal--Whittemore M, Patil S, Pareja F, Bowser ZL L, Dickler M, Norton L, Morrow M, Hudis C and Brogi E. Memorial Sloan Kettering Cancer Center, New York, NY and University of Kansas Medical Center, Kansas City, KS.

Body: Background: The 21-gene recurrence score (RS) estimates the likelihood of distant recurrence and the benefit from chemotherapy in patients with early-stage node-negative, estrogen receptor (ER)-positive, HER2-negative breast carcinoma. The use of the assay resulted in a substantial reduction in adjuvant chemotherapy usage. In this study, we reviewed the outcome of patients with node-negative, ER+/HER2- breast cancer and low recurrence score treated at our center to further verify the prognostic value of the assay.

Design: We identified breast cancer patients treated at our center between 09/2008 and 08/2013 with ER-positive, HER2-negative breast cancer and known RS. We reviewed clinicopathological characteristics, RS, treatment and outcome data. The Institutional Review Board approved the study.

Results: We identified 1406 consecutive patients with early stage node negative ER+/HER2- breast cancer and low RS [RS 0-10: 510 (36%), RS 11-17: 896 (64%)] in the study period. The median age at breast cancer diagnosis was 56 years (range 22-90). Sixty-three (4%) patients were <40 years old at breast cancer diagnosis. A total of 1362 (97%) patients received endocrine therapy, and 170 (12%) received chemotherapy. The median follow up time was 46 months (range 1-85). Six (0.4%) of the 1406 patients developed biopsy proven distant metastases within 5 years of breast cancer diagnosis, 5 of which were in the RS 11-17 group (Table 1). Three of the 5 patients with RS 11-17 and distant metastases were younger than 40 years old at breast cancer diagnosis. In the RS 11-17 group, the absolute incidence of distant metastases among patients with breast cancer diagnosed at age younger than 40 years old is 7.1% (3/42), whereas the absolute incidence of distant metastases among patients ≥40 years is 0.2% (2/854).

Conclusion: Our results suggest that young age (<40 years old) might be a negative prognostic factor even in patients with low RS. Analysis of data from other studies is necessary to further validate this observation.

Table 1. Clinicopathologic characteristics of the 6 patients with ER-positive, HER2-negative, node-negative breast carcinoma of recurrence score <18 who developed distant metastasis

Patients	#1	#2	#3	#4	#5	#6
Age at diagnosis (years)	50	54	37	71	38	39
Family history of breast/ ovarian cancer	No	Yes	No	No	No	Yes
Personal history of breast carcinoma	No	Ipsilateral DCIS	No	Ipsilateral DCIS	No	No
Tumor type	ILC	IDC	IDC	IDC	IDC	IDC
Tumor size (cm)	2.1	1.3	2.7	2.3	1.6	2.1
Tumor Grade	2	2	2	2	2	3
LVI	No	No	No	No	Yes	No
ER (%)	90	95	95	95	95	95
PR (%)	30	5	85	75	75	95
Oncotype DX RS	5	12	12	13	14	17
Surgery	BTM	TM	BTM	BCS	BCS	BTM
Radiation	No	No	No	Yes	Yes	No
Endocrine therapy	Yes	Yes	Yes	Yes	No	Yes
Chemo	No	No	CMF	No	No	No

Time interval to metastasis (months)	58	41	25	20	48	12
Site of metastasis	Bone	Multiple	Lung	Multiple	Multiple	Bone
Follow-up (months)	72	53	59	64	71	42
Survival	AWD	AWD	AWD	DOD	AWD	AWD

Abbreviations: RS, recurrence score; ILC, invasive lobular carcinoma; IDC, invasive ductal carcinoma; LVI, lymphovascular invasion; BTM, bilateral total mastectomy; TM, total mastectomy; BCS, breast conserving surgery; CMF, cyclofosphamide, metotrexate and 5-fluorouracil. AWD, alive with disease; DOD, died of disease.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-15

Title: Tumor and serum DNA methylation in women receiving preoperative chemotherapy (PST) with or without vorinostat in primary operable HER2-negative breast cancer in TBCRC008

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Body: Background:

DNA methylation is a promising prognostic and predictive biomarker of response to treatment in advanced breast cancer (Fackler MJ. *Can Res* 2014). We evaluated whether baseline and change in tissue and serum methylation would predict pathological complete response (pCR) in patients with HER2-negative early breast cancer treated with PST.

Methods:

TBCRC008 was a multicenter placebo-controlled trial that investigated pCR (no invasive cancer in breast/axilla) following 12 weeks of preoperative carboplatin and albumin-bound paclitaxel with or without the histone deacetylase (HDAC) inhibitor vorinostat in 62 patients with HER2-negative breast cancer. We performed an exploratory planned biomarker study correlating baseline and change (D15) in tumor tissue and serum methylation with pCR. Methylation was measured using cumulative methylation index (CMI) in quantitative multiplexed methylation-specific polymerase chain reaction (QM-MSP) assays; cMethDNA assay for serum (Fackler MJ. *Can Res* 2014) and QM-MSP for tissue (Fackler MJ. *Can Res* 2004). The analysis population included all patients with available methylation and pCR data. Association between CMI level (log transformed baseline, D15, change) and pCR was evaluated using univariate as well as multivariable logistic regression models controlling for treatment arm and estrogen receptor (ER) status. Additional subgroup analyses assessed association of CMI level with pCR stratified by ER status and treatment arms, respectively. Spearman's correlation coefficient was performed to evaluate the correlation between tissue and serum CMI.

Results:

pCR data were available for 61 of 62 patients. One patient with unknown response was treated as a non-responder. The pCR rate was similar in both arms (vorinostat 25.8%, placebo 29%). Both tissue and serum CMI data were available in 55 (baseline), 50 (D15) and 46 (change) patients. Elevated serum methylation based on a predefined threshold (6.9 units) was identified in 23/59 (39%) patients. In univariate analysis, one log unit increase in tissue CMI levels at D15 was associated with a 40% lower chance of obtaining a pCR (odds ratio, OR=0.60, 95% CI 0.37-0.97; p=0.037). The multivariable analysis did not show a statistically significant association of tissue or serum CMI with pCR, although a similar trend in magnitude towards association with pCR was observed for D15 and change in tumor tissue CMI (OR 0.67 and 0.57, p=0.129 and 0.147 respectively). Subgroup analyses suggested a significant association between tissue CMI levels at D15 and pCR in the group treated with vorinostat [tissue OR: 0.44 (0.20, 0.93), p=0.03; serum OR 0.37 (0.13, 1.07), p=0.07] but not in the placebo group. No significant correlation was observed between tissue and serum CMI.

Conclusion:

This is the first study to evaluate the predictive role of tissue and serum methylation (CMI) in patients with early breast cancer treated with PST ± an HDAC inhibitor. Methylation was detected more frequently in tissue than serum. Tissue CMI levels at D15 may predict poor response to this regimen. Further evaluation of the predictive role of methylation in early breast cancer is warranted.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-16

Title: Randomized study of COX2 inhibition on systemic inflammation in obese and non-obese subjects

Brenner AJ J, Lengfelder L, Quach DK K, Cavazos DA A, Ramirez RJ, Gruslova A, Kist K, Lathrup K, Kaklamani V, Beeram M and deGraffenried LA A. CTRC at UT Health Science Center San Antonio, San Antonio, TX; UT Austin, Austin, TX and START Center for Cancer Care, San Antonio, TX.

Body: Introduction: Obesity is associated with poor breast cancer outcomes in postmenopausal women. Our prior retrospective studies have shown that use of nonsteroidal anti-inflammatory drugs (NSAIDs) are associated with reduced recurrence in obese breast cancer patients and a doubling of time to recurrence. Because it was recently determined that CD163+ M2 macrophages were clinically associated with fast proliferation, poor differentiation, estrogen receptor negativity and histological duct type in human primary breast tumors, the mechanism proposed was a decrease in prostaglandin E2 (PGE2) and aromatase locally in the breast with a concomitant decrease in circulating M2-activated tumor associated macrophages (TAMs). **Methods:**

Postmenopausal women of varying body habitus were recruited at the CTRC in San Antonio and underwent randomized assignment to 1 of 3 arms: Aspirin (ASA) at 81mg daily, 1500mg of docosahexaenoic acid (DHA) and 2500mg eicosapentaenoic acid (EPA) given daily, or combined ASA and DHA/EPA. Sera were collected prior to and following 28 days of exposure, and cytokines including prostaglandin E2 were assessed via enzyme-linked immunosorbent assay (ELISA). 28 circulating cytokines/chemokines were assessed by Luminex array using Millipore Milliplex MAP to look for associations between cytokine array profiles, PGE2 production and macrophage activation. Circulating class M-1 activated and M-2 activated macrophages were enumerated by flow cytometry to assess how PGE2 modulation influences macrophage phenotype and function. Investigators were blinded to randomization until analysis was complete. **Results:** A total of 122 patients were randomized with 2 drop outs and 115 completing the 28 days of intervention as planned. The median BMI was 31.4, with 12.8% normal (BMI <25.0), 27.3% overweight (25.0-29.9), and 59.9% obese (>29.9). Patients had a median age of 63 (47-76), 91% white, and 46.0% Hispanic. A positive correlation was observed between BMI and baseline PGE2 levels. The most consistent impact on PGE2 was observed with ASA with 81% obtaining a decrease from baseline (median change -28%); by comparison 55.1% (-1%) and 65.6% (-22%) of subjects showed decrease in the DHA/EPA and combined groups respectively. As of today, full cytokine profiling was performed on a subset of 38 patients and revealed a positive correlation with change in PGE2 and cytokines: EGF, Eotaxin, GM-CSF, IL1Ra, IL5, IL8, MIP1b, and TNFa.

Conclusion: Aspirin alone most consistently impacted patient circulating PGE2 levels, and will be used in planned studies as an adjunct to adjuvant endocrine therapy in obese hormone receptor positive post-menopausal patients. Full cytokine and macrophage activation status will be reported.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-17

Title: EndoPredict multigene test in the prediction of response and outcome after neoadjuvant hormonal treatment with letrozole and palbociclib (OOTR-N007)

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Body: Background:

The EndoPredict [EP] is a multi-gene expression assay designed to predict the relapse-free survival of patients with estrogen receptor [ER] positive, HER2 negative breast cancer treated with adjuvant endocrine therapy alone. Relative expression levels of the eight prognostic genes [AZGP1, BIRC5, DHCR7, IL6ST, MGP, RBBP8, STC2, UBE2C] and 3 reference genes were analyzed by real-time PCR system. EP low-risk patients have a better RFS when treated with endocrine therapy. One study indicated that EP high-risk patients are more sensitive to anthracycline-based neoadjuvant chemotherapy than low-risk patient.

Palbociclib is an orally active potent and highly selective reversible inhibitor of CDK4/6. N007 study aims to evaluate the efficacy of the pre-operative use of letrozole plus palbociclib for ER positive, HER2 negative post-menopausal breast cancer. Whether EP could predict for response and outcome to such treatment will be evaluated.

Method:

Twenty patients were treated. EP assays were intended to be tested for all patients. RNA were extracted from paired FFPE tumour blocks [core biopsies and corresponding surgical sections]. EP score were calculated for the first 7 paired samples. Also the EP Clin Score which integrate both mRNA of prognostic genes expression and their pathological parameters, including tumor size and nodal status, were also evaluated. The change in EP score were compared with that of Ki67 and also with the pathologic tumor response.

Results:

The results of the first 7 patients were presented. The overall pCR rate rate was 14.3%[1/7]. 85.7% [6/7] samples were classified as low risk group. Paired T-test showed EP score is significantly lower [P=0.0014] after neoadjuvant hormonal treatment [NHT]. The change of EP score before and after treatment corresponds to the change in Ki67. Both have a similar prediction of response

Results

Subject	EP (before)	EP (after)	Ki67 (before)	Ki67 (after)	Change in EP	Change in Ki67	EP CLin
001	4.9	3.7	5%	1%	-1.2	.4%	2.7
002	8.2	4.7	50%	3%	-3.5	.47%	2.3
003	10	7.1	13%	13%	-2.9	0%	4.3
004	5.6	-	10%	-	-5.6	-100%	-
005	7.7	5.3	9%	1%	-2.4	-8%	2.8
006	3.7	2.2	20%	8%	-1.5	-12%	2.6
007	4.4	3.7	10%	5%	-0.7	-5%	3.4

. EP scores either remained high or showed minimal change for patients with stable disease [SD]. The subsequent EP Clin score was high for one of such patients, indicating high clinical risk. The corresponding Ki67 did not show any change and was low after treatment [Table1].

Conclusion:

pCR has been used as an endpoint for neoadjuvant chemotherapy trial. Our data suggest that the EP score can be reliably determined in core biopsy specimens and can be applied as endpoint on NHT trials. Changes in EP score correlated with that of Ki67 and also with response. However, changes in EP score may have a better prediction of response than Ki67. EP Clin score,

which integrates clinical parameters, may predict subsequent risk and clinical outcome after treatment. The data of all the 20 patients will be presented at the meeting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-18

Title: A clinically validated DNA microarray for high-resolution HER2 testing defines a new genomic subtype in high-risk breast cancer with equivocal results by IHC and FISH

Gunn S, Yaziji H, Sims C, Govender S, Moore M, Cotter P and Jones S. Targeted Genomics, San Antonio, TX; Vitro Molecular Laboratories, Miami, FL; PacificDx, Irvine, CA and Oncology Insights, Scottsdale, AZ.

Body: Background: In all stages of breast cancer, the HER2 status of a patient's tumor is critically important as both a prognostic indicator, and for predicting response to targeted anti-HER2 therapies. CAP/ASCO 2013 guidelines recommend that newly diagnosed, recurrent, and metastatic breast tumors be evaluated for HER2 positivity by protein-based immunohistochemistry (IHC) and/or chromosome-based fluorescence in situ hybridization (FISH). In the majority of cases, these testing modalities provide a clearly actionable “positive” or “negative” answer. However, in an estimated 10- to 20% of breast cancers, both tests are reported as “equivocal” leaving the clinician with a treatment decision dilemma and no definitive alternative testing method. Here we report validation of an IHC-targeted DNA microarray comparative genomic hybridization (array CGH) assay for HER2 equivocal breast cancer, and definition of a new genomic subtype of HER2 status in high-risk breast cancer with equivocal IHC and FISH results. **Methods:** IHC-targeted HER2 receptor “hot spot” DNA samples extracted from 25 formalin fixed paraffin embedded (FFPE) breast tumor tissue samples previously characterized by IHC and FISH, were analyzed by array CGH. Eight tumors were known to be highly HER2 positive, seven tumors had IHC scores of 0 with negative FISH, and ten tumors had HER2 receptor staining by IHC (1-2+) and equivocal results by FISH (4-6 HER2 gene copies.) Tumor DNA (test) and human genomic DNA (reference) were fluorescently labeled, and competitively hybridized to a custom-designed genomic DNA microarray with high-density probe coverage of the HER2 amplicon on chromosome 17 (Agilent Technologies, Santa Clara CA). The array design includes over 4,600 chromosome 17 probes representing the p arm, q arm, telomeric and centromeric regions with 66 tiling probes over the HER2 (ERBB2) gene. Following hybridization, average HER2 gene copy number was calculated for each tumor sample by converting mean log₂ signal intensity ratio value into genomic region copy number adjusted for % clonal fraction and experimentally established log₂ ratio compression of the assay. **Results:** 25/25 (100%) of samples yielded adequate DNA for analysis and all highly HER2 positive and HER2 negative results were confirmed by array CGH. In 10/10 IHC equivocal cases with HER2 gene copy number 4-6 by FISH, CGH results confirmed HER2-Low gene copy number.

Results for 25 Validation Samples

Number of Cases	IHC Score	FISH	CGH Copy Number	Result
8	3+	Positive	> 6	HER2-Positive
10	1-2+	Equivocal	4-6	HER2-Low
7	0	Negative	< 6	HER2-Negative

Conclusions: High-resolution HER2 testing by IHC-targeted DNA microarray analysis accurately classifies HER2 status in breast cancer and better defines the HER2-Low genomic subtype most often called “equivocal” by standard IHC and FISH testing. This subcategory is characterized at the protein level by IHC evidence of anti-HER2 therapy target receptor expression on the surface of the cells, and at the genomic level by HER2 gene copy number < 6. Results of the NSABP-B47 clinical trial and further studies with larger numbers of HER2-Low patients are needed to determine if these patients benefit from anti-HER2 therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-19

Title: Tumor RNA disruption index as a tool to predict response to neoadjuvant chemotherapy in breast cancer: Optimizing timing of biopsy

Samkari A, Chung W, Parissenti A, Pritzker L, Trabulsi N, Basik M and Boileau J-F. Jewish General Hospital, Montreal, QC, Canada; Northeast Cancer Centre, Sudbury, ON, Canada; Kingston General Hospital, Kingston, ON, Canada and Rna Diagnostics Inc., Toronto, ON, Canada.

Body: BACKGROUND. Early detection of tumor response to neoadjuvant therapy (NAT) could be used to tailor therapy and lower toxicity from ineffective treatments. The CCTG MA.22 trial has shown that RNA disruption is associated with breast cancer response to NAT when measured by image guided core biopsy mid-treatment. The objectives of this study were: 1) to determine the optimal time to measure the tumor RNA Disruption Index (RDI) after initiation of NAT when assessed by fine needle aspiration (FNA) in an office setting and 2) to determine if RDI could predict response to a second chemotherapy agent in patients that had a suboptimal response.

METHODOLOGY. We performed a prospective pilot study including patients with palpable biopsy-proven breast cancer eligible for NAT. Chemotherapy and surgery were at the discretion of the treating physician. Two FNAs after cycles 1, 2, 3 and after initiation of a new chemotherapy agent were collected in RNA Protect Cell Reagent and sent to Rna Diagnostics Inc. to assess RDI. Prospectively recorded clinical tumor measurements and surgical pathology reports were obtained. Tumor pathological response (pR) after NAT was measured by pathological complete response (pCR: no invasive disease in breast) and residual cancer burden index (RCBI).

RESULTS. 30 patients were accrued to the study. One patient withdrew consent, one patient was found to have metastatic disease and did not undergo surgery, and one patient had bilateral breast cancer (n= 29 evaluable tumors). ER+HER2-: 38% (11/29), ER-Her2-: 28% (8/29) and HER2+: 34% (10/29). 89% (25/28) of patients received taxane and anthracycline containing regimens. All HER2+ received trastuzumab. Our pCR and RCBI 0-1 rates were 24% (7/29) & 38% (11/29) respectively. At cycles 1, 2 and 3, RDI could be evaluated in 72% (21/29), 73% (16/22) and 44% (7/16) of palpable tumors. After the switch to a new agent, RDI could only be evaluated in 30% (3/10) of patients with palpable tumors. Using a tumor RDA cutoff at 5 (non-responder RDI < 5 (NR) and responder RDI ≥ 5 (R)), responder status between cycle 1 and 2 was concordant in 73% (11/15). After 1 cycle, NR vs. R was associated with numerically lower pCR (13% (2/15) vs. 33% (2/6), p=0.54) and RCBI 0-1 at surgery (20% (3/15) vs. 33% (2/6), p=0.60). These findings were similar after cycle 2. Non-analyzable samples (NAS) because of absence of RNA were associated with high pR (pCR: 38% (3/8) and RCBI 0-1: 75% (6/8)). The 3 NR at cycle 1 that achieved a significant pR had either non-palpable tumors or NAS after switching to a new chemotherapy agent.

CONCLUSION. RDI can be measured by FNA in an office setting and could be helpful to identify early non-responders to NAT. The optimal time to perform RDI is after 1 or 2 cycles of treatment, which should be considered in ongoing and future trials. This study was underpowered to detect a statistically significant correlation between RDI and pR. NAS is associated with high pR and could represent responders to treatment. This early data suggests that RDI is unlikely to be helpful in assessing response to a second chemotherapy agent after receiving 4 cycles of standard chemotherapy. The use of RDI to tailor NAT needs to be evaluated in larger prospective trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-20

Title: An optimized workflow to analyze *ESR1* mutations in both circulating cell-free and circulating tumor cell DNA by digital PCR

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Body: *Background*

In metastatic breast cancer (MBC) patients *ESR1* mutations (*mESR1*) in cell-free DNA (cfDNA) have been related to endocrine therapy (ET) resistance. Such mutations might also be detectable in circulating tumor cells (CTCs). Mutation detection in small amounts of cfDNA and in CTCs in a background of leukocytes is highly challenging. The current study evaluated how to reliably investigate *mESR1* status in such minute amounts of cfDNA and in DNA from CellSearch-enriched CTCs.

Materials & Methods

Plasma (200 μ L) and matched CellSearch-enriched CTC fractions of 7 healthy blood donors (HBD) and 29 MBC patients at baseline and after ET (≥ 5 CTC/7.5 mL) were evaluated. cfDNA was isolated from plasma with the QIAamp CNA kit and CTC-enriched DNA with the AllPrep kit (Qiagen). *mESR1* status in both cfDNA and CTC-enriched DNA fractions was compared with or without whole genome amplification (repli-g WGA, Qiagen) or *ESR1* target specific amplification. Quantitative PCR (qPCR) for wild type (WT) *ESR1* was used to control the number of WT copies loaded into the chips for digital PCR (dPCR) analysis. The variant allele frequencies (VAF) of hotspot mutations for *ESR1* (D538G, Y537S, Y537C and Y537N) were evaluated with mutation-specific Taqman assays by chip-based dPCR (QuantStudio 3D, Thermo Fischer Scientific).

Results

To allow inclusion of as many samples as possible, we successfully downscaled the volume of required plasma from 1 mL to 200 μ L as this resulted in the same VAF. Sample-type specific thresholds for *mESR1* presence were established (2% for the cell-free plasma samples, at which percentage all HBDs were negative, and 0.5% for the CTCs to allow identification of one mutated CTC-specific copy in a background of $\sim 1,000$ leukocytes).

WGA was unable to adequately amplify fragmented cfDNA, resulting in a too low DNA yield. However, locus-specific target pre-amplification of a 136 bp fragment covering all 4 different mutations followed by mutant specific dPCR performed well for both cfDNA and CTC DNA, but only if the loading of the pre-amplified product into the dPCR chips was optimized by qPCR for the number of WT *ESR1* copies.

The most optimal results for dPCR data interpretation were obtained after: 1. including at least one positive sample in each dPCR session; 2. using a “safe loading window”, 3. loading and reading chips at least twice in QuantStudio 3D ; 4. critically evaluating the contribution by a non-specific “comet effect”; and 5. after loading the data in the software, performing at least two independent data analyses to exclude intra-observer variations.

Summary

Here we describe our workflow to assess *mESR1* in a limited amount of plasma cfDNA or CellSearch enriched CTC DNA. This workflow has been successfully used to investigate the *mESR1* VAF status in DNA from matched CTC DNA and cfDNA of MBC patients before start of 1st line endocrine therapy and at progression (see also abstract number 851017).

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-09-21

Title: Clinical evaluation of miR-100 as a predictor of endocrine-responsiveness in hormone-receptor positive breast cancer

Zucchini G, Petrelli A, Kubatzki F, Cargnelutti M, Di Virgilio MR, Sarotto I, Martincich L, Ponzzone R, Martinello R, Sapino A, Nuzzo A, Giordano S and Montemurro F. Candiolo Cancer Institute-FPO (IRCCS), Candiolo, Italy.

Body: Background: Micro RNAs (miRNAs) are short, non-coding RNA molecules that act as negative regulators of gene-expression, mainly at the post-transcriptional level. Alterations in miRNA functions have been implicated in a variety of human diseases, including cancer

We demonstrated that the ectopic expression of miR-100 in cancer stem cells (CSCs) derived from aggressive, basal-like BC (HRs and HER2 negative) caused loss of stemness and the acquisition of a hormone receptor positive and endocrine treatment sensitive phenotype (Petrelli et al, *Oncotarget* 6;2315-30, 2015). We therefore sought to study whether miR-100 is a determinant of the endocrine-responsive phenotype in HR-positive BC patients (pts).

Methods: Women with newly diagnosed, estrogen-receptor and/or progesterone-receptor positive, HER2 negative BC were eligible for this study. Treatment consisting of tamoxifen for pre-menopausal and letrozole for post-menopausal pts was administered daily for 21 days (+/- 3 days) before breast surgery. MiR-100 levels in pre-treatment tumor biopsies, measured as fold-change with respect to a reference RNA and transformed to the natural logarithms to normalize the data, were correlated with proliferative response to endocrine therapy, as measured by Ki67 expression in the final surgical specimen. The primary end-point was a complete proliferative response (CPR), defined as a post-treatment Ki67 \leq 1%. Additionally, we considered a "post-hoc" composite endpoint where response was defined as a post treatment Ki67 <10% together with a Ki67 reduction \geq 80% compared to pre-treatment values. The target accrual is 88 patients (pts). Here we report the results of the first interim analysis focusing on post-menopausal pts receiving letrozole.

Results: A total of 42 pts were evaluable for miR-100 levels and response to endocrine therapy. Median ER and PgR expression was 99% (58%-99%) and 96% (0-99%) respectively. Median pre-treatment Ki67 was 18% (5-76%). Thirty-one tumors were ductal carcinomas, 9 were lobular and 2 were "other" histotypes. The median (range) miR-100 values in pre-treatment specimens was 2.253 (0.460-3.750). After treatment, median Ki67 was 4% (1%-46%) and the median percentage variation with respect to baseline values was -74% (0% to -94%). A CPR was observed in 5/42 pts (12%, 95% C.I. 5%-25%). The median miR-100 levels in responders and non-responders were 3.058 and 2.198, respectively (p = 0.03). Logistic regression analysis showed that each unit increase in miR-100 was associated with a 7-fold increase in the likelihood of a CPR (OR 7.056, 95% C.I. 1.103-45.141, p = 0.04).

Considering the composite end-point, 17/42 pts (40%, 95% C.I. 27%-56%) were considered responders. Median miR-100 levels in responders and non-responders were 2.427 and 1.956, respectively (p = 0.05).

Conclusions: preliminary results of this prospective clinical trial suggest that miR-100 can be a modulator of the endocrine-responsive phenotype in post-m pts with HR-positive breast cancer. The study is completing its target accrual and an investigation of miR-100 targets is being conducted.

GZ and AP contributed equally to this work.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-01

Title: Utilization and outcomes of post-mastectomy radiotherapy in women with 1-3 positive axillary nodes following neoadjuvant chemotherapy: A multiply-imputed, propensity-adjusted National cancer database analysis

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Body: Background: Use of post-mastectomy radiotherapy (PMRT) following neoadjuvant chemotherapy (NAC) varies widely. The combined analysis of NSABP B1724 studies suggests a higher risk of locoregional recurrence for women with persistently positive lymph nodes following NAC. The impact of PMRT on survival in the subset of patients with 1 to 3 positive nodes after NAC is not clear and the goal of the current study is to evaluate practice patterns and the impact of PMRT on survival in this subset of patients.

Methods: The National Cancer Database was queried for women who underwent NAC followed by mastectomy and had 1-3 persistent axillary lymph nodes with or without adjuvant PMRT. A propensity score was generated to account for indication bias under two circumstances: one with the original dataset and one following a multiple imputation to complete the dataset to allow for a more accurate propensity score generation. Factors impacting utilization of PMRT were calculated with backwards-selection binary regression. Kaplan-Meier with log-rank test and Cox-regression analyses were used for survival.

Results: Within the queried cohort of 14,895 women from 2006-2012, 70% of women received PMRT following NAC with 1-3 positive axillary lymph nodes. One lymph node was positive in 56%, 2 in 25.9%, and 3 in 18.1%. PMRT was utilized in 67.3% of women with 1 node positive, 71.5% with 2 nodes positive, and 76.2% with 3 nodes positive. Factors associated with the usage of PMRT were: residence on the east coast in a highly populated region with proximity to the treatment center, later year of diagnosis (71% of women in 2012), younger age, non-African American race, non-Hispanic descent, private insurance, higher clinical stage, triple negative/high grade histology, pathologic T4, greater number of axillary node positive, positive margins or LVSI, greater number of nodes examined, and use of adjuvant hormonal therapy (all $p < .05$).

Median follow up was 40.1 months (range: 2.6-106.9 months). Prior to multiple imputation, a propensity score was generated which accounted for 28.7% of cases. PMRT did not have an impact on survival within the limited analysis. In a pooled propensity-adjusted Cox analysis following multiple imputation to complete the dataset, PMRT was found to have an OS HR of 0.878 (95%CI 0.806-0.957, $p = .003$). Five year overall survival rates with and without PMRT were 77.6% and 75.4% ($p < .001$). For 1-3 positive nodes respectively, 5-year overall survival rates with and without PMRT were: 79.7% vs. 79.1% ($p = .041$), 77.3% vs. 70.8% ($p < .001$), and 71.8% vs. 68.0% ($p = .004$).

Conclusions: In a population-based analysis of women who underwent neoadjuvant chemotherapy prior to mastectomy, post-mastectomy radiotherapy appears to confer a survival advantage in women with 1-3 residual axillary lymph nodes. There continues to be a wide variation in practice nation-wide with a significant number of women with persistently positive 1-3 axillary lymph nodes not receiving PMRT.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-02

Title: A signature predictive of early vs. late recurrence after radiation treatment (RT) for breast cancer that may inform the biology of early, aggressive recurrences

Speers C, Chang L, Santola A, Liu M, Zhao SG G, Chandler B, Olsen E, Bartelink H, Feng FY Y and Pierce LJ J. University of Michigan Hospital and Health System, Ann Arbor, MI and Netherlands Cancer Institute, Amsterdam, Netherlands.

Body: Purpose: Unmet clinical needs in breast cancer (BC) management include the identification of patients (pts) at high risk to fail locally despite standard local therapy including RT and understanding the biology of these recurrences. We previously reported¹ a RT response signature and here extend those studies to identify a signature predictive of timing of recurrence after completion of RT (before or after 3 years).

Methods: Two independent patient cohorts (treated with BCS) from non-randomized clinical trials were used for training and validation. The training cohort included 119 pts with in-breast tumor recurrence and the validation cohort had 25 pts with recurrences. Initial feature selection used Spearman's rank correlation correlating gene expression (14,806 genes) to recurrence time. Genes with sig. correlation (FDR <0.1) and large expression range (fold change >2) were used to train an elastic net penalized Poisson regression model. This model was locked and then applied to the validation dataset. Cox regression was used for both univariate and multivariable analyses (UVA and MVA). To identify biological-related concepts, Spearman's corr. coefficients of recurrence time to gene expression within the training cohort were used to generate a pre-ranked list upon which GSEA pathway analysis was performed.

Results: Spearman's correlation identified 485 genes whose expression was significantly associated with recurrence time (early vs. late). Feature reduction further refined the gene list to 41 genes, which were retained within the signature and locked for further validation. In the training dataset the Spearman's correlation of the continuous score to recurrence time was 0.852 with a P-value of 1.3×10^{-34} and an AUC of 0.92. Application of this early vs late signature to an independent BC validation set accurately identifies pts with early vs. late recurrences (Spearman's corr.=0.537, p-value<0.007, AUC=0.74, sensitivity=0.71, specificity=0.73, PPV=0.77, NPV=0.67). In UVA and MVA the early vs. late recurrence signature remained the most significant factor associated with recurrence time. Although independent of intrinsic subtype, GSEA analysis of the 41 genes retained within the signature identifies proliferation and EGFR concepts associated with early recurrences and luminal and ER-signaling pathways associated with late recurrences. Knockdown of genes associated with the early and late recurrences is currently underway to assess phenotypic changes (proliferation and clonogenic survival as a measure of early and durable RT response) associated with the early and late recurrence-associated genes.

Conclusion: In this study we derive a BC-specific RT signature predictive of early vs. late recurrence with biologic relevance and validate this signature for prediction of timing of recurrence in an independent clinical dataset. By identifying pts with tumors likely to recur sooner vs. later this signature has the potential to allow for a furthered understanding of the biology underlying early and late recurrences and has a potential to personalize RT, particularly in patients for whom treatment intensification is needed.

1. Clin Cancer Res. 2015 Aug 15;21(16):3667-77.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-03

Title: A randomized trial of accelerated breast radiotherapy utilizing either 3-dimensional radiotherapy versus intensity modulated radiotherapy

Leonard CE E, Sobus RD D, Fryman S, Sedlacek S, Kercher J, Widner J, Asmar L, Wang Y, Howell K, Barke L and Carter D. Rocky Mountain Cancer Center, Denver, CO; Rocky Mountain Cancer Center, Aurora, CO; SurgOne, Littleton, CO; Linasmar Consulting, Houston, TX and Invision Sally Jobe Breast Network, Greenwood Village, CO.

Body: PURPOSE: Primary objective: patient self-assessment of breast pain between 3-dimensional radiotherapy (3D-CRT) versus intensity modulated radiotherapy (IMRT). Secondary objectives: breast cosmesis as well as local-regional recurrence and survival statistics.

METHODS AND MATERIALS: 656 patients (3D-CRT n=325; IMRT n=331) were prospectively randomized to either IMRT or 3D-CRT accelerated partial breast radiotherapy to 38.5 Gy in 10 BID 3.85 Gy fractions. Follow-up was: 1, 4, 8, 12, 16, 20, 24 months then yearly. At follow-up, patients completed a cosmesis/pain self-assessment form and physicians completed a cosmesis and disease-status form.

RESULTS: 636 patients completed treatment (3D-CRT n=316; IMRT n=320). Median age was 62. Mean tumor size was 1.1 cm. Mean margin was 7mm. Histology was: 74.5% IDCA, 7% ILCA, 17% DCIS, 0.5% Tubular, 1% Mucinous. 99% were ER+. HER2/*neu* status by IHC was 3+ in 16% of patients. Median follow-up is 2 years. Tables 1 and 2 show there is no significant difference in patient-assessed pain and cosmesis between the two treatment arms (p=0.14, =0.68 respectively). Decreasing pain and worsening cosmesis as reported by the patient were significantly related to time (p<0.01, =0.012 respectively). MD assessed cosmesis worsened significantly from baseline in the IMRT compared to 3D-CRT cohort (p=0.045). At 2 years Grade 3 and 4 toxicities were 1.5% and 3.9% respectively for 3D-CRT versus IMRT cohorts. Overall Survival at 2 years were 99.7% for both cohorts. There were 3/319 (0.9%) and 7/328 (2.1%) ipsilateral breast recurrences in the 3D-CRT and IMRT cohorts respectively.

Patient breast pain by follow-up interval

12 Months (3D n=167 and IMRT n=163)					
Rx Modality	None	Mild	Moderate-Severe	p value	
3D	50.9%	47.3%	1.8%	0.44	
IMRT	52.1%	44.2%	3.7%		
24 Months (3D n=111 and IMRT n=109)					
3D	52.3%	47.7%		0.07	
IMRT	66.1%	33.9%			
36 Months (3D n=50 and IMRT n=34)					
3D	60.0%	40.0%		0.37	
IMRT	58.8%	41.2%			
48 Months (3D n=12 and IMRT n=12)					
3D	25.0%	75.0%		0.19	
IMRT	58.3%	41.7%			
Results from mixed model for pain grade					
Effect	Estimate	SE	Lower	Upper	p value
3D vs IMRT	0.081	0.055	-0.026	0.189	0.14
Visit (Baseline, 12, 24, 36, 48 month)	-0.101	0.019	-0.137	-0.064	<0.01

Table 1.**Patient breast cosmesis by follow-up interval**

12 Months (3D n=162 and IMRT n=158)					
Rx Modality	No change	Slight change	Obvious change	Drastic change	p value
3D	40.1%	38.3%	19.1%	2.5%	0.83
IMRT	41.8%	40.5%	15.2%	2.5%	
24 months (3D n=108 and IMRT n=108)					
3D	38.9%	40.7%	18.5%	1.9%	0.30
IMRT	41.7%	29.6%	26.9%	1.9%	
36 Months (3D n=50 and IMRT n=34)					
3D	38.0%	34.0%	28.0%	0%	0.37
IMRT	32.4%	35.3%	26.5%	5.9%	
48 Months (3D n=10 and IMRT n=12)					
3D	10.0%	30.0%	60.0%		0.10
IMRT	50.0%	8.3%	41.7%		
Patient breast cosmeses by follow-up interval					
Effect	Estimate	SE	Lower	Upper	p value
3D vs IMRT	0.026	0.064	-0.100	0.153	0.68
All Visit (Baseline, 12, 24, 36, 48 month)	-0.053	0.021	-0.095	-0.012	0.01

Table 2.

Conclusion: There were no significant differences in patient-assessed pain and cosmesis between the two treatment arms ($p=0.14$, $=0.68$ respectively) and no significant increase in pain over time. However, MD assessed cosmesis showed worsening cosmesis in the IMRT cohort compared to the 3D-CRT cohort when compared to baseline.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-04

Title: Accelerated partial breast irradiation versus whole breast irradiation: Health-related quality of life analysis from a phase 3 trial

Meattini I, Saieva C, Desideri I, Miccinesi G, Francolini G, Meacci F, Muntoni C, Scotti V, De Luca Cardillo C, Marrazzo L, Simontacchi G, Pallotta S, Sanchez L, Casella D, Bernini M, Orzalesi L, Nori J, Bianchi S and Livi L. University of Florence - Azienda Ospedaliero Universitaria Careggi, Florence, Italy and Cancer Risk Factor Branch, Cancer Research and Prevention Institute (ISPO), Florence, Italy.

Body: Background. Accelerated partial breast irradiation (APBI) represents a valid option for selected early breast cancer (BC); potential advantages of APBI include shorter treatment time, improved safety profile, and a cost reduction compared with standard fractionation.

We reported the final analysis of quality of life (QOL) results from a phase 3 randomized trial comparing standard adjuvant radiotherapy (50 Gy in 25 fractions, plus 10 Gy boost) to APBI using IMRT technique (30 Gy in 5 daily fractions). The 5-year results have been recently published showing equivalence in terms of local control (ClinicalTrials.gov, NCT02104895).

Methods. Overall 205 patients (105 APBI and 100 WBI) fully completed the given questionnaires at time 0 (RT start), time 1 (RT end), and time 2 (2-year follow up). Patients were asked to complete two specific questionnaires on QOL, the EORTC QLQ-C30 as a reliable and valid measure of the QOL of cancer patients in multicultural clinical research settings, and the BR23 module as a supplementary questionnaire for assessing QOL issues relevant to patients with BC. The statistical software SPSS (SPSS Inc, Chicago, IL, USA) for Windows (version 22), and STATA (StataCorp LP, College Station TX77845, USA) for Windows (version 12) were used. Chi-squared test or Mann Whitney U test were used to compare the individual characteristics of the patients between two arms. Mean and standard deviations (SD) were calculated for all QOL domains, and all scores were compared between APBI and WBI arms using the Mann Whitney test due to non-parametric distribution of data. The Kruskal-Wallis test was used to compare the scores between age groups.

Results. Mean values (and SD) of QLQ-C30 scores according to arm in the series of 205 BC patients at time 2 (time 0 vs time 2), showed significant improvement in favor of APBI in terms of global health status (mean 75.5 vs 59.5, SD range 13.3-22.0; $p < 0.0001$), main functional ($p < 0.01$), and symptom scales ($p < 0.01$). Concerning the BR23 module, APBI showed significantly better outcome in terms of body image perception (mean 89 vs 72.1, SD 13.2-26.6; $p < 0.0001$) and future perspective (84.8 vs 57, SD 23.1-28.5; $p < 0.0001$) among functional scales; breast (6.1 vs 18.9, SD 6.6-18.2; $p < 0.0001$) and arm symptoms (11.7 vs 19.6, SD 13.4-19; $p = 0.002$) among symptom scales.

Conclusions. Women treated with APBI reported a significantly better QOL outcome as compared with women treated using WBI. QOL improvement was evidenced in terms of functional, symptoms, and global health status/QOL scales, both at the end of radiation and at a 2-year follow-up time.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-05

Title: Omitting radiotherapy in women ≥ 65 years with early breast cancer and favorable histopathology after breast-conserving surgery, sentinel node biopsy and adjuvant endocrine therapy is safe

Villman KKA KA, Wickberg Å, Killander F, Lindman H, Bjöhle J, Edlund P, Tennvall-Nittby L, Bachmeier K, Carlberg M, Blomqvist C, Ahlgren J and Liljegren G. Örebro University Hospital, Örebro, Sweden; Skåne University Hospital, Lund University, Lund, Sweden; Akademiska Hospital, Uppsala University, Uppsala, Sweden; Karolinska Institute and University Hospital, Stockholm, Sweden; Gävle Hospital, Gävle, Sweden; Skåne University Hospital, Lund University, Malmö, Sweden and Karlstad Central Hospital, Karlstad, Sweden.

Body: Background: The benefit of radiotherapy in older women with endocrine responsive early breast cancer treated with breast-conserving surgery and endocrine therapy is unclear. The aim of this study was to verify if omission of radiotherapy in a predefined cohort of patients with good prognosis early breast cancer after breast conservation is safe.

Methods: Eligibility criteria were: consecutive patients with age ≥ 65 years, breast-conserving surgery (sector resection + sentinel node biopsy), clear margins, unifocal T 1 N0, Elston grade 1 and 2, estrogen receptor-positive. After informed consent adjuvant endocrine therapy, either tamoxifen or an aromatase inhibitor, was prescribed for 5 years. Primary endpoint was ipsilateral breast tumor recurrence (IBTR). Secondary endpoints were contralateral breast cancer, recurrence-free survival (RFS) and overall survival (OS).

Results: Between 2006 and 2012, we included 603 women from 14 Swedish centers. Two patients did not fulfill the inclusion criteria and were excluded from the analysis. Median age was 71 years (range 65 to 90). At a median follow-up of 59 months (range 2 to 110) 13 IBTR (cumulative incidence at five years, 1.3% (95% CI, 0.6% to 2.7%), 4 regional recurrences (one combined with IBTR), 2 distant recurrences both without IBTR or regional recurrence and 11 contralateral breast cancers was observed. Twenty-nine patients were diagnosed with tumors of other origin. Seven of them were endometrial cancers. There were 39 deaths. Only one of the deaths (2.6%) was due to breast cancer and 11 (28.2%) were due to other cancers (2 endometrial cancers). Five-year overall survival was 93.9% (95% CI, 91.4% to 95.7%).

Conclusion: This study demonstrates, with a median follow-up of 59 months, that breast-conserving surgery and endocrine therapy without radiotherapy is a safe treatment option in women with early breast cancer and favorable histopathology aged ≥ 65 years. The risk of IBTR is comparable to the risk of contralateral breast cancer. The low rate of breast cancer deaths indicates that breast cancer mortality is of secondary importance in this subset of women.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-06

Title: Hypofractionated nodal radiotherapy (RT) did not increase arm morbidity compared to conventional fractionated nodal RT

Leong N, Truong P, Tankel K, Kwan W, Weir L and Olivotto I. Saskatchewan Cancer Agency Allan Blair Cancer Centre, Regina, SK, Canada; BC Cancer Agency Vancouver Island Centre, Victoria, BC, Canada; Alberta Health Services Tom Baker Cancer Centre, Calgary, AB, Canada; BC Cancer Agency Fraser Valley Centre, Surrey, BC, Canada; BC Cancer Agency Vancouver Centre, Vancouver, BC, Canada and Alberta Health Services Cross Cancer Institute, Edmonton, AB, Canada.

Body: Purpose: Regional nodal radiation therapy (RT) can cause adverse arm symptoms and lymphedema. Hypofractionation (HF), defined as >2 Gy/fraction, improves convenience but whether it increases arm morbidity is unclear. This study evaluates patient-reported arm symptoms in women treated with HF compared to conventional fractionation (CF) RT (defined as ≤ 2 Gy/fraction).

Materials / Methods: Provincial cancer registries were used to identify subjects who received 3D, CT-planned nodal RT for pT1-3 pN0-2 M0 breast cancer from 2007-2009 in British Columbia and 2008 – 2010 in Alberta, Canada. Treatment eras were selected to enable sufficient follow-up time to develop late arm symptoms. Following research ethics approval, eligible patients were mailed an explanation letter and an externally validated, Self-reported Arm Symptom Scale (SASS) survey. The SASS included 8 questions about arm symptoms, with responses on a 5-point Likert scale regarding arm/hand problems (numbness, pain, stiffness, immobility and swelling), and 5 questions related to activities of daily living (ADL). Clinicopathologic characteristics and SASS scores were compared between HF vs. CF nodal RT cohorts using non-parametric analysis (on ordinal and scale responses) and binned chi-squared analysis (comparison for responses of 1 vs. > 1).

Results: 800/1759 eligible patients returned a completed survey (45.5%). Upon detailed chart review of responders, 92 cases with recurrence or metastasis were excluded. The remaining 708 cases formed the study cohort. Of these, 406 (57%) patients received HF RT (modal dose/fractionation 40 Gy/15 fractions (fx) and 45 Gy/20 fx), and 302 (43%) received CF RT (45 Gy/25 fx, 48-50 Gy/25 fx, and 50.4 Gy/28 fx). A boost was delivered to the breast in 22% of subjects, equally by fractionation group ($p=0.31$).

Median time interval since RT completion was 5.67 years. The mean age at diagnosis was 59.0 in HF vs 53.8 years in CF-treated cohorts ($p<0.001$). The mean # positive ($n=3$) and excised ($n=12$) nodes were similar between fractionation cohorts ($p=0.44$). Primary tumor size was marginally larger in the CF group (2.8 vs. 2.7 cm, $p=0.03$). 42.9% of patients were treated with partial mastectomy with no significant difference in fractionation ($p=0.54$). Overall, 602 (75.3%) patients received chemotherapy. A trend toward increased use of CF after chemotherapy was observed (78.8% vs. 72.7%, $p=0.07$).

The mean sums of responses for the arm symptoms / ADL components of the SASS were 12.5 / 7.6 vs. 13.3 / 7.9 for the HF and CF groups respectively ($p=0.17$ / 0.85). On analysis of individual questions, the CF group had a higher prevalence of self-reported symptoms, including shoulder stiffness ($p=0.04$), trouble moving the arm ($p=0.02$), and ability to reach overhead ($p<0.01$). There was no difference in self-reported arm swelling between the two groups ($p=0.57$).

Conclusion: Hypofractionated nodal RT was not associated with an increase in patient-reported arm symptoms or disability compared to conventional fractionated nodal RT. Subjects treated with CF RT reported more disability in certain aspects of arm and shoulder function. These data support the use of shorter fractionation when the regional lymph nodes are part of the therapeutic target.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-07

Title: Prospective longitudinal study of epidermal thickening in breast cancer patients treated with conventional versus hypofractionated radiotherapy

Torres MA A, Yang X, Mister D, Ali A, Kahn S and Liu T. Glenn Family Breast Center, Winship Cancer Institute, Emory University, Atlanta, GA.

Body: Purpose: We conducted two prospective longitudinal studies of epidermal thickening in breast cancer (BRCA) patients treated with either conventional (CRT) or hypofractionated radiotherapy (HRT) using ultrasound images to objectively measure radiotherapy (XRT)-induced skin changes. **Methods:** Following breast conserving surgery, 105 consenting Stage 0-IIIA BRCA patients were enrolled on two studies of whole breast XRT without regional nodal irradiation. In the first, 66 subjects were treated with conventional fractionation (50 Gy plus a sequential 10 Gy boost at 2 Gy per fraction). In the second, subsequent study, 39 patients with characteristics under-represented in trials of HRT were treated to 39.9 Gy at 2.66 Gy per fraction with a simultaneous integrated boost of 8.1 Gy at 0.54 Gy per fraction. Inclusion criteria for this trial included breast separation >25cm, age <50 years, chemotherapy treatment, or non-Caucasian race. Prior to XRT, the last week of XRT, and 12 weeks and 1 year post XRT, subjects underwent objective ultrasound measurements of epidermal thickness over all four quadrants of the treated breast. A skin thickness ratio (STRA) was generated normalizing for corresponding measurements taken of the untreated breast. Pertinent patient, tumor, and treatment characteristics were collected at all time points, as well as peripheral blood for gene expression assays and assessment of inflammatory markers. **Results:** HRT patients were significantly younger than CRT patients [mean age 52 (38-73) versus (vs.) 56 years (26-75), $p=0.03$]. There were no other significant differences in patient, tumor, or treatment characteristics. Baseline measurements indicated 68% of CRT (mean increase 27%, SD 0.30) vs. 71% of HRT patients (mean increase 23%, SD 0.29) had skin thickening in the treated versus untreated breast prior to XRT ($p=0.50$). At all subsequent time points, CRT patients had significantly higher mean STRA than HRT patients [1.53, SD 0.48 vs. 1.33, SD 0.34 during RT ($p=0.03$); 1.62, SD 0.48 vs. 1.31, SD 0.45 at 12 weeks post RT ($p=0.002$); and 1.45, SD 0.39 vs. 1.27, SD 0.40 at 1 year post RT ($p=0.03$), respectively]. At 1 year, 31% of CRT and 38% of HRT patients had an STRA which was either less than or equal to baseline, indicating full recovery. In multivariable analysis, baseline STRA ($p<0.001$), BMI ($p=0.002$), chemotherapy ($p=0.004$) and CRT treatment ($p=0.02$) predicted for higher STRA 1 year post XRT. Dmax and breast volume receiving 107% of the dose did not predict for STRA. Gene ontology analysis revealed an over-representation of genes, differentially regulated among patients with high and low STRA, involved in the immune and defense responses. Tumor necrosis factor 2 receptor levels were also significantly higher in CRT than HRT treated patients at 1 year ($p=0.03$). **Conclusions:** Our findings indicate that CRT is associated with higher and more severe changes in STRA up 1 year after XRT than HRT, even among patients perceived to be at high risk for XRT-induced skin toxicity and who were not well-represented in the original HRT trials. An increase in peripheral markers of inflammation may be one mechanism by which CRT, BMI, and chemotherapy are associated with higher STRA 1 year post XRT.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-08

Title: Factors affecting the administration of post-mastectomy radiation therapy (PMRT) in Michigan

Gorski DH H, Braun T, Munir K, Griggs JJ J, Breslin TM M and Henry NL Lynn. Wayne State University School of Medicine, Detroit, MI; Barbara Ann Karmanos Cancer Center, Detroit, MI; University of Michigan, Ann Arbor, MI and Northwestern University Feinberg School of Medicine, Chicago, IL.

Body: Background: Evidence-based guidelines for locoregional therapy of invasive breast cancer treated with mastectomy include adjuvant PMRT for: ≥ 4 positive axillary lymph nodes (LN); T3 or above; or a positive surgical margin. We assessed PMRT uptake using data from the Michigan Breast Oncology Quality Initiative (MiBOQI), a Blue Cross Blue Shield of Michigan/Blue Care Network-sponsored collaborative quality initiative, and identified factors influencing its use in Michigan.

Methods: We prospectively collected clinical data on all patients with stage I-III breast cancer in 25 health systems belonging to MiBOQI and identified patients who underwent mastectomy from 2008 to 2013. Patients with previous cancer, bilateral disease, or treated with neoadjuvant chemotherapy were excluded. Univariate and multivariate analyses were performed to identify independent factors associated with the use of PMRT in patients with 0, 1-3, and 4+ positive LNs. Covariates included age, hormone receptor status, HER2 status, surgical margin, T category, Charlson comorbidity index, and immediate reconstruction. Two-tailed p-values < 0.05 were considered significant. Analyses were carried out using SAS software, version 9.4 (SAS Institute, Cary, NC).

Results: We identified 6,596 patients with stage I-III invasive breast cancer. Of these, 4,455 had no positive axillary LNs; 1,481, 1-3 positive LNs; and 660, ≥ 4 positive LNs. There was wide variation in PMRT use across MiBOQI sites, from 13% to 63.% in patients with 1-3 positive LNs (overall 42%) and from 35% to 91% in patients with 4+ positive LNs (overall 69%). In multivariate analyses stratified by nodal status (0, 1-3, 4+), age ≥ 70 yrs was negatively associated with PMRT. We also noted lower PMRT use in women aged 51-69 with 0 and 1-3 positive LNs (Table 1).

Table 1. Multivariate analysis: PMRT and age

Age	(+)LNs = 0		(+)LNs = 1-3		(+)LNs ≥ 4	
	Odds ratio	p-value	Odds ratio	p-value	Odds ratio	p-value
≤ 50 y (reference)	1.0		1.0		1.0	
51-69 y	0.70 (0.49 - 0.98)	0.03	0.69 (0.54 - 0.88)	< 0.0001	1.03 (0.63 - 1.65)	< 0.0001
≥ 70 y	0.60 (0.37 - 0.96)		0.26 (0.18 - 0.37)		0.31 (0.18 - 0.54)	

In the 0 and 1-3 positive node groups, PMRT use was strongly associated with T category and close or positive margin status (Table 2).

Table 2: Surgical characteristics and PMRT uptake

VARIABLE	(+)LNs = 0		(+)LNs = 1-3		(+)LNs ≥ 4	
	Odds ratio	p-value	Odds ratio	p-value	Odds ratio	p-value
Margin status						
Negative (reference)	1.0		1.0		1.0	
Positive	16.7 (10.5 - 26.7)	< 0.0001	2.27 (1.32 - 3.90)	0.001	0.69 (0.32 - 1.47)	0.90
Close (< 1 mm)	4.63 (3.03 - 7.10)		1.90 (1.29 - 2.80)		0.97 (0.55 - 1.69)	

T category						
T0 to T2 (reference)	1.0		1.0		1.0	
T3 to T4	26.2 (16.4 - 41.8)	<0.0001	3.24 (2.19 - 4.78)	<0.0001	0.86 (0.57 - 1.28)	0.45

Finally, there was no association between PMRT use and hormone receptor status, HER2 status, Charlson comorbidity index, or reconstructive surgery at the time of mastectomy.

Conclusions: PMRT use across Michigan was lower than the American College of Surgeons Commission on Cancer target of 90% in patients with 4 or more positive LNs. Contrary to common belief, immediate reconstructive surgery was not independently associated with decreased PMRT uptake. Understanding reasons for nonuse of PMRT may lead to interventions to increase its use by MiBOQI member institutions in patients for whom it is indicated.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-09

Title: Delineation of internal mammary nodal target volumes in breast cancer radiotherapy

Jethwa KR R, Kahila MM M, Hunt KN N, Brown LC C, Corbin KS S, Park SS S, Yan ES S, Boughey JC C and Mutter RW W.
Mayo Clinic, Rochester, MN.

Body: Purpose/Objectives: The optimal clinical target volume (CTV) for internal mammary node irradiation (IMNI) is uncertain in an era of increasingly conformal volume-based treatment planning for breast cancer. We mapped the location of gross internal mammary lymph node (IMN) metastases in order to identify areas at highest risk for harboring occult disease.

Methods and Materials: Patients with axial imaging of IMN disease, including fluorodeoxyglucose F-18 (FDG18) positron emission tomography (PET-CT) and magnetic resonance imaging (MRI), were identified from a breast cancer registry. The IMN location was transferred by a radiation oncologist and breast radiologist onto the corresponding anatomic position on representative axial CT images of a patient in the treatment position. Distribution of lymph nodes, and their location was compared with consensus group guidelines of IMN target delineation.

Results: Sixty-seven patients with 130 IMN metastases were mapped. The location was in the first three intercostal spaces in 102 of 130 (78%) nodal metastases. Eighteen of 130 (14%) IMN were located caudal to the third intercostal space, while 10 of 130 (8%) IMN were located cranial to the first intercostal space, including 3 patients with isolated IMN metastases at that location in the absence of distant disease. Of the 102 nodal metastases within the first three intercostal spaces, 54 (53%) were located within the RTOG consensus volume. Relative to the internal mammary (IM) vessels, 19 (19%) nodal metastases were located medially with a mean distance of 2.2 mm (SD 2.9 mm), while 29 (28%) were located laterally with a mean distance of 3.6 mm (SD 2.5 mm). Ninety and ninety-five percent of lymph nodes within the first three intercostal spaces would have been encompassed within a 4 mm and 6 mm medial and lateral expansion on the IM vessels, respectively.

Conclusions: For women with indications for elective IMNI, a 4 mm medial and lateral expansion on the IM vessels within the first 3 intercostal spaces may be appropriate. In women with known IMN involvement, cranial extension to the confluence of the IM vein with the brachiocephalic vein +/- caudal extension to the 4th or 5th interspace with a 6 mm medial and lateral expansion may be considered, provided that normal tissue constraints are met.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-10

Title: Risk factors of locoregional recurrence, locoregional failure pattern and role of postmastectomy radiotherapy for T1-2 breast cancers with 1-3 positive axillary lymph nodes

Wang S, Peng R, Jin J, Wang W, Song Y, Liu Y, Liu X, Yu Z, Fang H, Ren H and Li Y. Cancer Hospital, Peking Union College and Chinese Academy of Medical Sciences, National Cancer Institute, Beijing, China.

Body: Objective: To evaluate risk factors of locoregional recurrence (LRR), the locoregional failure pattern, and the role of postmastectomy radiotherapy (PMRT) for T1-2 breast cancers with 1-3 positive axillary lymph nodes.

Material and methods: 1348 patients with T1-2 breast cancer and 1 to 3 positive lymph nodes treated with mastectomy and without neoadjuvant systemic therapy during 1999-2010 were retrospectively analyzed. Six hundred and thirty (46.7%) patients were T1 and 709 (52.6%) were T2. Six hundred and sixty-seven (49.5%), 417 (30.9%) and 264 (19.6%) patients have 1, 2 and 3 axillary positive lymph nodes. After mastectomy, 235 (17.4%) received radiotherapy, 1249 (92.7%) received adjuvant chemotherapy, 958 (73.5%) received adjuvant hormonal therapy, and 39 (2.9%) received adjuvant trastuzumab. Overall survival (OS) and locoregional recurrence free survival (LRFS) rates were calculated by Kaplan-Meier method and the differences were compared by log-rank test. Cox logistic regression analysis was performed.

Results: With a median follow-up time of 79 months (range, 12-194 months), totally 155 patients died, 127 of whom died of breast cancer, 23 died of other illness or accident, 8 died of unknown reasons. Ninety-eight patients had LRR, 60 of them were isolated LRR. The 5- and 10-year cumulative LRFS were 95.7% and 91.6%, respectively. The 5-year LRFS rates were not statistically different between patients with or without PMRT (95.9% vs 94.5%, $p = 0.338$). However, patients treated with PMRT had more high-risk factors including T2 diseases, PR negative, 3 positive nodes, positive node ratio more than 10%, non-luminal A subtype than those without PMRT. Multivariate analysis showed that patient who did not receive PMRT had a significant decreased LRFS compared with those who did ($p = 0.037$, HR = 2.519, 95% CI 1.058 - 6.000).

For 1113 patients who did not receive PMRT, there was no significant difference in LRFS between different molecular subtypes, the 5-year LRFS rates were 98.0%, 94.2%, 96.4% and 92.8% for patients with luminal A, luminal B, Her2+, and triple negative subtype ($p=0.129$). On multivariate analysis, significant factors associated with decreased LRFS were T2 stage ($p = 0.014$, HR = 2.146, 95% CI 1.170-3.936), PR negative ($p = 0.017$, HR = 2.651, 95% CI 1.189-5.911) and percentage of positive lymph node (PLN) >10% ($p = 0.026$, HR = 0.532, 95% CI 0.305-0.928). In patients with 3 risk factors treated without PMRT, the 5-year and 10-year locoregional recurrence risk (LRR) was both 15.7%. Among patients who had LRR, 48.1%, 35.9%, 13.2% and 2.8% had supraclavicular, chest wall, axilla and internal mammary node recurrence, respectively.

Conclusion: T1-2 breast cancer patients with 1-3 positive axillary lymph nodes had an excellent prognosis in modern treatment era, with a 10-year LRR risk less than 5%. Risk factors of decreased LRFS included T2 stage, PR negative and PLN >10%. PMRT should be considered for patients with all 3 risk factors. Supraclavicular nodal region and chest wall should be routinely irradiated.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-11

Title: Prone vs. supine positioning for optimizing radiation heart exposure during left breast radiotherapy: A simple clinical tool for prediction*

Kahán Z, Gaál S, Cserhádi A, Rárosi F, Boda K and Varga Z. University of Szeged, Szeged, Csongrád, Hungary and University of Szeged, Szeged, Csongrád, Hungary.

Body: The benefit of reduced radiation heart exposure in the prone vs. supine position, individually differs according to the anatomical features of the patient (Varga Z, Int J Radiat Oncol Biol Phys. 2009;75:94-110). Preferable positioning may be predicted by a statistical model. This validated calculator using the patient's body mass index (BMI), the median distance of the anterior surface of the left coronary artery (LAD) from the chest wall (D_{med}) and the heart area (A_{heart}) included in the radiation field in a single CT scan at the middle of the heart (*median plane*, P_{med}) provides quantitative estimates of the dose differences to the LAD or heart in the two positions (Varga Z, Acta Oncol. 2014;53:58-64).

In this prospective cohort study, the goal was to develop a reliable method to collect data for the calculator without acquiring a full series of CT scans in both positions. The study was approved by the Institutional Review Board of the University of Szeged, and all the enrolled patients gave their written informed consent to participation. Eligible patients needed postoperative left breast radiotherapy. In 100 patients, a single CT slice image representing the middle of the heart (reference plane, P_{ref}) was acquired by using the AP scout view in the supine position for the selection of the transversal plane appropriate for the measurements of D_{med} and A_{heart} . Thereafter, CT series were acquired in both positions, and analyses were performed on 1. the conformance of the P_{ref} with P_{med} , 2. the effect of plane miss on the choice of preferable position. 3. Finally the sensitivity and specificity of this simple clinical method was reevaluated based on the dosimetry data (mean LAD dose, V25Gy heart) obtained using the topogram for selecting the position.

In 55 cases, P_{ref} was the same as P_{med} , while in 27 and 18 cases, P_{ref} and P_{med} differed by 2 or more planes, respectively. No difference was found between the values of D_{med} or A_{heart} as measured in P_{ref} vs. P_{med} , independently whether P_{ref} was identified correctly or not. The suggestion on treatment position per P_{ref} measurements, was correct in 85 cases, was ambiguous in 8 cases and incorrect in 7 cases. Sensitivity of the calculator based on P_{ref} measurements was 90% (both mean LAD dose and V25Gy heart), while specificity was 72% (mean LAD dose) and 68% (V25Gy heart). The simple clinical method was tested in additional 60 left breast radiotherapy cases, by taking a topogram and a CT series in the suggested position only. Mean LAD dose (mean [CI95%] prone vs. supine: 6.5 [5.7-7.3] Gy vs. 7.5 [5.6-9.5] Gy) and V25Gy heart (mean [CI95%] prone vs. supine: 0.87 [0.67-1.11] % vs. 1.13 [0.55-1.71]%) well corresponded to the institutional dose constraints based on dosimetry data achieved with individual positioning in >300 patients with left-sided breast cancer.

We consider this simple clinical tool appropriate for assisting individual positioning aiming at maximum heart protection during left breast irradiation.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-12

Title: Radiation therapy in 1-3 node positive mastectomy patients: Who benefits?

Ellis ED D, Scanlan JM M, Kaplan HG G, Kieper DA A, Morris AD D and Atwood M. Swedish Center for Research and Innovation, Seattle, WA; Providence Health and Services, Seattle, WA and Swedish Cancer Institute, Seattle, WA.

Body: Introduction A recent meta-analysis on radiation therapy (XRT) demonstrated improved outcomes and recommended its use. However, these data were derived from the 1960's-1980's and might not reflect the effects of XRT when used in modern oncology practice [1]. Consequentially, XRT benefits for mastectomy patients with 1–3 positive nodes at the time of surgery (SX) remain uncertain. In this retrospective study we examined XRT effects in a modern cohort of mastectomy patients with 1–3 positive nodes at SX, across multiple outcomes: loco-regional recurrence (LRR), distant recurrence (DR), total recurrence (TR), breast cancer (BCa mortality) and all-cause mortality (ACM).

Subjects The Swedish Cancer Institute's breast cancer patient registry was used to identify mastectomy patients who had 1-3 positive lymph nodes at SX and known HER-2 receptor status. HER-2 positive patients who did not receive Herceptin were excluded.

Methods Clinical, pathological, treatment and outcomes data were extracted from our registry. Logistic multiple regressions were used to identify clinical and pathology elements available at the time of SX that correlated with LRR, DR, TR, BCaM and ACM. Regression model elements included: tumor receptor status - estrogen (ER), progesterone (PR) and HER-2, presence or absence of lymphovascular invasion (LVI), extension of LVI, number of LN+, node positivity ratio (positive/examined), surgical margins (SxM); patient age, chemotherapy, XRT and hormonal treatment.

Results The application of these filters to our breast cancer registry yielded 935 patients with a mean follow up time of 7 years. Our sample was “modern”: 95% of our patients were diagnosed after 1999, 80% after 2004. Logistic regression indicated that across all patients, XRT was associated with improved LRR (Nagelkerke R²=5%, p<.01), and ACM (Nagelkerke R²=1%, p<.05). The four clinical and pathologic elements that most strongly correlated with outcomes were LVI (+), LN+>1, PR (-) and SxM (+) and we stratified the population into risk groups based on the number of factors present; low-risk = no factors, medium-risk = 1 factor, High-risk = 2+ factors. We also stratified by treatments, comparing the effects of chemotherapy alone vs. chemotherapy plus XRT across the major patient outcomes and risk groups (see Table 1). This comparison only showed XRT effects for LRR in the higher risk groups.

XRT effects by Risk Groups

	TREATMENT	N=	LRR	DR	TR	BCa Mort.	ACM
Low Risk	CH+, No XRT	108	0%	4.6%	4.6%	2.9%	3.7%
Group	CH+, XRT+	41	0%	2.5%	2.5%	2.4%	2.5%
Medium Risk	CH+, No XRT	215	2.8%	10.2%	12.1%	5.6%	10.7%
Group	CH+, XRT+	188	0% *	8.5%	8.5%	4.3%	6.4%
Highest Risk	CH+, No XRT	83	4.8%	8.4%	13.3%	8.4%	16.9%
Group	CH+, XRT+	122	.8% +	13.1%	13.9%	8.2%	13.1%

* =p<.05, + =p<.07

Discussion XRT improved LRR in the medium and high risk groups, but lacked benefit in the low-risk group and did not provide statistically significant improvements in BCa survival. These results suggest caution in using XRT in low-risk patients, but it has some value in reducing LRR in medium and high-risk populations, without survival benefits.

1. Lancet Volume 383, No. 9935, p2127–2135, 21 June 2014.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-13

Title: Can the risk of radiation-induced cardiac disease in breast cancer be mitigated with cardiac-sparing techniques?

Smith TL L, Mokhtech M, Bradley JA A, Lightsey JL L, Morris CG G and Mendenhall NP P. University of Florida College of Medicine, Gainesville, FL and University of Florida Health Proton Therapy Institute, Jacksonville, FL.

Body: Introduction: Data suggest that long-term survivors of breast cancer treated with radiation therapy (RT) have an increased risk of cardiac events despite an overall survival benefit, particularly women with left-sided breast cancers. We hypothesized that with cardiac-sparing radiation techniques there would be no difference in long-term cardiac risks between women with right- and left-sided breast cancers.

Materials and Methods: The outcomes of 775 consecutive women treated between 1984 and 1999 with breast-conserving therapy (BCT) (n=424) and post-mastectomy radiation therapy (PMRT) (n=351) for stage 0-3 breast cancer were assessed retrospectively through a review of medical records and contact with living patients. The choice of BCT vs PMRT was based on tumor board disposition and patient preference; mastectomy was recommended for multicentric or T3 breast cancers. Ninety-six percent of all node-positive patients received treatment to all nodal regions, including the internal mammary (IM), axillary (AX), and supraclavicular (SC) nodes. BCT patients had computed tomography-planned tangential breast radiation with photons with IM nodes treated within the tangent fields or with a separate en face electron field to minimize cardiac exposure. PMRT patients were treated with en face electron fields for the chest wall and IM for sparing of the lung and heart. The AX and SC nodes were treated with a matched anterior photon field with a posterior-anterior boost field (PAB) when necessary to achieve adequate dose in the AX with both BCT and PMRT. Overall, 411 patients were node-negative and 353 were node-positive. All patients have a minimum potential follow-up of 16.4 years; median actual follow-up for the BCT group was 15 years (range, 0.1-31.2) and for the PMRT group it was 9.5 years (range, 0.1-30.5).

Results: Overall survival (OS), cause-specific survival (CSS), and freedom from local-regional recurrence (FFLR) rates for the cohort at 15 years were 58.2%, 72.0%, and 90.8%, respectively. Rates of freedom from cardiac events (FFCE), pulmonary events, and second malignancy were 87.6%, 93.6%, and 86.3%, respectively. On multivariate analysis, OS was correlated with stage (p=.045), number of positive nodes (p=.002), age (p<.0001), diabetes (p =.0021), and modality (p=.0017). Not surprisingly, 15-year survival for patients treated with BCT was better than for patients treated with PMRT (70.9% vs 59.7%; p<.0001). CSS was associated with stage (p=.0207), number of positive nodes (p=.0409), and modality (p=.0003). FFLR was associated with number of positive nodes (p=.0484). FFCE was associated with pretreatment cardiac disease (p<.0001), stage (p=.0461), and age (p=.0056), but not with either breast cancer laterality (p=.1906) or modality (BCT vs PMRT; p=.7487).

Discussion: Long-term disease control and survival outcomes were better in BCT than PMRT patients, likely due to selection criteria. Cardiac events were associated with pretreatment heart disease, older age, and stage, suggesting that this population may benefit from advanced radiation techniques that can further limit cardiac dose. Neither breast cancer laterality nor treatment modality was associated with cardiac events.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-14

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-15

Title: Pentoxifylline and vitamin E therapy compliance in patients with breast fibrosis following adjuvant radiotherapy

Famoso JM M, Gonzalez VJ J and McBride A. University of Arizona, Tucson, AZ and University of Arizona.

Body: Purpose: Radiation therapy is an important treatment modality in the curative management of early stage breast cancer. With increasing cancer survivorship, late effects of cancer treatment have become increasingly relevant. Breast fibrosis is a common late effect following therapeutic irradiation which can potentially result in pain, poor cosmesis, and functional impairment. Randomized trials have suggested that radiation fibrosis may be reversible or preventable using a medication regimen of Pentoxifylline and Vitamin E. These clinical trials have shown excellent patient compliance and tolerability of this regimen, but it is unclear if this tolerability translates to clinical practice. The goal of this study is to investigate the clinical tolerability and compliance with pentoxifylline and Vitamin E therapy in patients with radiation induced breast fibrosis while determining possible correlates to discontinuation.

Methods: We identified ninety patients who were prescribed Pentoxifylline (400mg three time daily) and Vitamin E (400 IU once daily) following definitive breast radiation therapy from January 2013 to December 2015. A retrospective cohort study was conducted utilizing medical record analysis. Data was collected including patient age, comorbid conditions, concurrent medications, duration of pentoxifylline and Vitamin E therapy, any dose adjustments, patient reported side effects, and any reported reason for discontinuation. Multivariate analysis of correlation between medication compliance and these categorical variables was assessed with Chi squared (χ^2) analysis of independence.

Results: Raw patient compliance with Pentoxifylline and Vitamin E therapy was found to be poor with 33 of 87 (38%) of patients requiring either dose reduction or discontinuation of therapy due to side effects. The majority of patient complaints leading to dose adjustments were GI related with nausea (n=20) abdominal pain (n=4), and diarrhea (n=1) reported. There was a statistically significant relationship between concurrent antiemetic therapy and successful completion of pentoxifylline regimen. Patients completed therapy at a rate of 76% with concurrent antiemetic vs 48% without (p=0.007). There was a statistically significant relationship between concurrent proton pump inhibitor therapy and discontinuation of pentoxifylline. Patients completed pentoxifylline therapy at a rate of 33% with concurrent proton pump inhibitor vs 81% without (p=<0.001). Treatment compliance was not significantly correlated with age, pre-existing GERD, time since completing radiation therapy, concurrent use of aromatase inhibitor, tamoxifen, vitamin E, or chemotherapy.

Conclusion: Patient compliance with Pentoxifylline and Vitamin E for radiation induced breast fibrosis appears to be worse in clinical practice compared with previously reported randomized trials. Nausea was the most commonly reported side effect resulting in discontinuation of the regimen. Our data suggests concurrent antiemetic therapy is correlated with strong regimen compliance while concurrent proton pump inhibitor therapy is correlated with poor compliance independent of comorbid conditions. These data should be discussed with patients prior to initiation of treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-16

Title: Standardization of nodal radiation therapy (RT) through changes to a breast cancer clinical pathway (CP) in a large, integrated comprehensive cancer center network

Gebhardt BJ J, Horne ZD D, Heron DE E and Beriwal S. University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Body: Purpose

The efficacy of adjuvant RT following breast conserving surgery (BCS) in reducing risk of local recurrence is established. The results of Z11, AMAROS and MA-20 studies led to wide variation in RT treatment volume for patients (pts) who have sentinel node biopsy positive disease. CP's are a mechanism of standardizing care when many therapeutic options exist and clinical practice varies unnecessarily. We sought to evaluate the impact of changes to a CP guiding adjuvant RT in sentinel node positive pts for invasive breast cancer (IBC) on practice patterns throughout a large cancer network.

Methods

In 2003, we implemented a CP for management of IBC with adjuvant RT. In 2009, we required entry of management decisions into an online tool integrated with medical records to track CP choices and subject off-pathway selections to peer-review. The CP for treatment of pts with positive sentinel lymph nodes (LN) following BCS was modified in February 2015 to promote uniform treatment of regional LN irradiation (RNI). In summary, the CP recommended modified tangents (MT) including level 1 and 2 nodes for all pts with micrometastases. For pts with macrometastases, CP recommended including level 1 and 2 LN in MT and add additional field to include level 3, supraclavicular LN +/- IM node for pts with any adverse factor present defined as T2 disease, LVSI, high grade, ER negative, ECE or premenopausal pts. Data from treatment decisions entered into the support tool from June 2009 to April 2016 were obtained.

Results

From 2009 until CP modification in 2015, 1089 treatment decisions were entered. Decisions were heterogeneous and included 24 distinct options. Following pathway amendment, 178 decisions were entered. Three pts were enrolled in clinical trials & 9 referred to other providers and excluded. Of the remaining 166 pts, 7 (4.2%) were treated off pathway for poor performance status, patient preference, or gross residual disease. The 159 (95.8%) on-pathway pts were analyzed. Median age was 61 (range 32-90) years. All pts underwent BCS for IBC and had positive sentinel LN. Forty-four (27.7%) pts had micrometastatic LN disease, and 115 (72.3%) had macrometastases, of which 82 (71.3%) had adverse risk factors. All 44 pts with micrometastases were treated with MT. Six (5.2%) pts with macrometastases were treated with WBT RT, 33 (28.7%) with MT, & 76 (66.1%) with MT and a 3rd field. Seventy-six (92.7%) pts with adverse risk factors were treated with MT and 3rd field ($p < 0.001$).

Conclusions

CP's are useful tools for translating published research, national guidelines, and institutional experience into standardized patient management plans to promote evidence-based care and eliminate unnecessary variations in practice patterns that lead to inefficiency and inferior outcomes. Recognizing that our CP for adjuvant treatment of patients with positive sentinel LNs undergoing BCS allowed heterogeneous treatment selections, we modified the CP in 2015 based upon the latest evidence regarding RNI. We found that following the amendment, patients received RT fields guided by the CP leading to more standardized treatment based upon clinical risk factors and facilitating tracking of patient outcomes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-17

Title: Radiotherapy associated with concurrent bevacizumab in patients with non-metastatic breast cancer

Dautruche A, Belin L, Cottu P, Bontemps P, Lemanski C, De La Lande B, Baumann P, Missohou F, Levy C, Peignaux K, Reynaud-Bougnoux A, Denis F, Gobillion A, Ady Vago N, Fourquet A and Kirova Y. Institut Curie, Paris, France; Institut Curie, Paris, France; Institut Curie, Paris, France; CHU Jean Minjoz, Besançon, Doubs, France; Institut Régional du Cancer de Montpellier, Montpellier, Hérault, France; Institut Curie, Rene Huguenin Hospital, Saint-Cloud, Hauts-de-Seine, France; Centre d'Oncologie de Gentilly, Nancy, Meurthe-et-Moselle, France; Centre Henri Becquerel, Rouen, Seine-Maritime, France; Centre François Baclesse, Caen, Calvados, France; Centre Georges-François Leclerc, Dijon, Côte-d'Or, France; CHU Tours, Tours, Indre-et-Loire; Centre Jean Bernard, le Mans, Sarthe, France and Roche SAS, Boulogne-Billancourt, Hauts-de-Seine.

Body: Purpose/Objectives

The purpose of this study was to determine early and late toxicities among patients with non-metastatic breast cancer (BC) receiving concurrent bevacizumab (BV) and radiation therapy (RT).

Materials/Methods

Multicentre, prospective study, of the toxicity of adjuvant concomitant association of BV and RT in patients with non-metastatic BC enrolled in Phase 3 BEATRICE, BEVERLY and BETH trial. Early and late toxicities were assessed by the Common Terminology Criteria for Adverse Events v. 3.0 during RT, 12 months and 36 months after its completion.

Results

Sixty-four patients were included from October 2007 to August 2010. They all received adjuvant RT and BV concomitant treatment, plus neo-adjuvant BV for 24 patients. RT was adjuvant and normo-fractionated. Twelve months toxicity was available for 60 patients and 36 months toxicity was available for 43 patients. Median follow-up was 46 months (18-77). Median age was 51 years old (23-68). Among 63 evaluated patients during RT, acute radiation dermatitis was observed in 48 (76%) patients: Grade 1 for 27 (43%), grade 2 for 17 (27%), grade 3 for 4 patients (6%). Grade 2 acute oesophagitis was observed in 1 patient. At 3 years, few toxicities were observed: 6 patients (14%) had grade 1 pain, 4 (9%) had grade 1 fibrosis, one (2%) had grade 1 telangiectasis, one (2%) had grade 1 paresis, 3 (7%) had grade 1 lymphoedema and one grade 3 lymphoedema. No grade 4 toxicity was observed. At 12 months, only one evaluated patient had a LVEF <50% and none at 36 months.

Conclusions

Concurrent bevacizumab with locoregional RT is associated with acceptable early and late 3-years toxicities in patients with BC. Determination of late toxicity at 60 months is currently underway.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-18

Title: The use of hypofractionated radiotherapy for the treatment of women with early breast cancer in the Brazilian public health system may increase access to treatment: Cost effectiveness and budget impact analyses

Andrade TRdM Raquel de Moraes, Nazário ACP Celso Pinto and Fonseca MCM Cunio Machado. Federal University of São Paulo, São Paulo, Brazil.

Body: **OBJECTIVES:**To evaluate the cost effectiveness and economic impact of increasing the use of hypofractionated radiotherapy for the treatment of women older than 50 years, with early breast cancer(stages I and II) within the Brazilian National Health System(SUS).**METHODS:**Several studies show no difference concerning efficacy and safety between hypofractionated and conventional radiotherapy for the treatment of women older than 50 years with stages I and II disease.We built a cost-effectiveness Markov model in Excel which quantifies the cost and the amount of photon beams linear accelerator time used for the treatment of patients using hypofractionated(2.67Gy/fraction) or conventional(2Gy/fraction).The time horizon is 5 years.The perspective of the study is the SUS.The effectiveness was measured as amount of hours saved using hypofractionated in relation to conventional radiotherapy.Costs related to treatment are from DATASUS,and considered the cost of planning the radiotherapy, of the check-film and the use of photon beams linear accelerator.The discount we applied was 5% for costs and benefits.We performed univariate and probabilistic sensitivity analyses. The treatment fraction time was set as 15 minutes. We also built, in Excel, a budget impact model to simulate the increasing adoption of hypofractionated radiotherapy instead of conventional radiotherapyin the treatment of Brazilian women older than 50 years with early breast cancer.The model compares hypofractionated radiotherapy(2.67Gy/fraction) with conventional radiotherapy(2 Gy/fraction).We determined the number of women over 50 years with stages 1 and 2 breast malignant neoplasm that underwent adjuvant radiotherapy in 2013 and 2014, and then projected these populations for the years 2016 to 2020.We considered the costs of planning the radiotherapy, using the photon beams linear accelerator, and performing the check-film.We considered a yearly increase of 20% in the adoption of hypofractionated radiotherapy for the years 2016 to 2019(2016 20%;2017 40%;2018 60%;2019 80% and 2020 90%).The treatment fraction time was set as 15 minutes. **RESULTS:** The use of hypofractionated radiotherapy at 5 years was able to decrease the number of hours of treatment(-21,835hours) and the total cost of treatment (-\$11,790,229.64).The technology is cost saving.Based on the budget impact analysis, the annual incremental impact would be of -\$243,202.65, -\$490,294.13, -\$741,085.61, -\$995,388.73 and -\$1,127,712.81 providing 3,378, 6.810, 10,294, 13,826 and 15,664 free hours of the linear accelerator for the years 2016, 2017, 2018, 2019 and 2020, respectively.These photon beams linear accelerator free hours may allow 613, 1,380, 2,306, 3,392 and 4,010 additional patients to have access to breast cancer treatment during the years of 2016 to 2020 respectively. **CONCLUSIONS:**Considering the conditions proposed in these models increasing the use of hypofractionation as a radiotherapy technique to treat women older than 50 years, with early breast cancer within seems to increase the system efficiency saving money, optimizing the treatment schedule and providing access to treatment for more patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-19

Title: Two-year follow-up results of a multi-center trial of intra-operative electronic brachytherapy during breast conservation surgery for early stage breast cancer

Syed AMN, Chang H, Schwartzberg BS S, Bremmer AK K, Boylan S, Lopez-Penalver C, Vito CA A, Davis M, Dooley WC C, Chakravarthy AB B, Croomer C, Proulx GM M, Golder SL L, Ivanov O, Fernandez KL L, Farha M, Gonzalez V, Wengler C, Bhatnagar A, Neuner G, Kopkash K, Rahman S, Corn C, Costa P, Ellenhorn J and Cox C. Todd Cancer Institute / Long Beach Memorial Medical Center, Long Beach, CA; David Geffen School of Medicine at UCLA / Revlon/UCLA Breast Center, Los Angeles, CA; Sarah Cancer Research Institute/Rose Medical Center, Denver, CO; BREASTLINK, Murietta, CA; Sentara Northern Virginia, Woodbridge, VA; Doctors Hospital, Miami, FL; City of Hope National Medical Center, Duarte, CA; Swedish Medical Center, Englewood, CO; Oklahoma University, Oklahoma City, OK; Vanderbilt University, Nashville, TN; Staten Island University Hospital, Staten Island, NY; Exeter Hospital, Exeter, NH; Shannon Cannon Cancer Center at Parkridge Medical Center, Chattanooga, TN; Florida Hospital, Orlando, FL; MEDSTAR Health (Franklin Square), Baltimore, MD; MEDSTAR Health (Union Memorial), Baltimore, MD; University of Arizona, Tuscon, AZ; Martin Health System, Stuart, FL; Cancer Treatment Services, Casa Grande, AZ; Greater Baltimore Medical Center, Baltimore, MD; Rush University Medical Center, Chicago, IL; Diablo Vallen Oncology Hematology Medical Group, Pleasant Hill, CA; Phoenix Baptist Hospital, Phoenix, AZ; PORTO, Senhora Da Hora, Portugal; Tower Outpatient Surgery Center, Los Angeles, CA and University of South Florida, Tampa, FL.

Body: Objective

To assess the safety and efficacy of single-fraction, intra-operative radiation therapy (IORT) delivered with the Xofig[®] Axxent[®] Electronic Brachytherapy System[®] (eBx[®]) immediately following surgical resection for treatment of early stage breast cancer.

Methods

This phase 4, open-label, single-arm, prospective, non-randomized trial is still enrolling participants and is currently being conducted at 26 hospitals in the USA (25) and Portugal (1). 878 participants with biopsy-proven ductal carcinoma in situ (DCIS) or invasive ductal carcinoma who met the inclusion criteria underwent lumpectomy followed by single-fraction IORT to the lumpectomy cavity. Briefly, a small, presterilized lead shield piece was placed on the chest wall to reduce the dose to the ribs, and then a balloon applicator, suitable to the surgical bed, was placed in the lumpectomy cavity and inflated with saline (30-75 cc); skin was temporarily closed over the balloon and ultrasound was used to confirm a balloon surface-to-skin distance ≥ 1.0 cm. The Xofig System was used to deliver the 20 Gy dose at the balloon applicator surface. The balloon was deflated, lead shield and balloon removed and the surgical site sutured. The prespecified primary outcome of this 10-year follow-up study is recurrence of ipsilateral breast tumor at 5 years. Prespecified secondary outcomes include 10-year recurrence and cosmesis (Harvard Scale). Trial Registry: ClinicalTrials.gov; Identifier: NCT01644669.

Findings

Of the 878 participants treated, 877 participants received the prescribed 20 Gy dose with a mean radiation treatment time of 594.5 seconds, whereas one participant received 14 Gy due to a source failure. 569 participants have reached 18-month (333), 2-year (199), and 3-year (37) follow-up. The mean age at enrollment was 65 years (range 41-90). 219 participants had DCIS and 658 had invasive ductal carcinoma. The DCIS nuclear grade was high (N=79), intermediate (N=100), or low (N=40). Invasive cancers were Grade 1 (N=282), 2 (N=282), or 3 (N=94). 664 participants had T1 lesions, 56 had T2 lesions, and 3 were unknown. The mean tumor size was 12.33 ± 10.5 mm. Cosmesis was excellent to good in 90% of participants who reached 2-year follow-up. To date, 155 reported adverse events were Grade 2 or higher. The most frequent side effects were breast pain, seroma, induration, and erythema. There were nine deaths, none of which were breast cancer related, four ipsilateral breast recurrences, and three new contralateral breast cancers.

Conclusions

Early results from this multi-center trial demonstrate that IORT using the Xofig Axxent eBx System at the time of breast conservation surgery continues to be a promising treatment option for early stage breast cancer. The short course of radiation therapy for select patients has excellent to good cosmetic results and a low rate of high-grade adverse events and recurrences.

Funding

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2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-20

Title: A multi-center trial of intra-operative electronic brachytherapy during breast conservation surgery for early stage breast cancer: Early results of unplanned boost participants

Syed AMN, Chang H, Schwartzberg B, Bremner A, Boylan S, Lopez-Penalver C, Vito C, Davis M, Dooley W, Chakravarthy AB, Coomer C, Proulx G, Golder S, Ivanov O, Fernandez K, Farha MJ, Gonzalez V, Wengler C, Bhatnagar A, Neuner GA, Kopkash K, Rahman S and Costa P. Todd Cancer Institute, Long Beach Memorial Medical Center, Long Beach, CA; David Geffen School of Medicine at UCLA, Revlon/UCLA Breast Center, Long Beach, CA; Sarah Cancer Research Institute at Rose Medical Center, Denver, CO; Breastlink, Murietta, CA; Sentara Northern Virginia, Woodbridge, VA; Doctors Hospital, Miami, FL; City of Hope National Medical Center, Duarte, CA; Swedish Medical Center, Englewood, CO; Oklahoma University, Oklahoma City, OK; Vanderbilt University, Nashville, TN; Staten Island University Hospital, Staten Island, NY; Exeter Hospital, Exeter, NH; Sarah Cannon at Parkridge Medical Center, Chattanooga, TN; Florida Hospital Department of Surgery, Orlando, FL; Medstar Franklin Square Medical Center, Baltimore, MD; Medstar Union Memorial Hospital, Baltimore, MD; University of Arizona, Tucson, AZ; Martin Health System, Stuart, FL; 21st Century Oncology, Casa Grande, AZ; Greater Baltimore Medical Center, Baltimore, MD; Rush University Medical Center, Chicago, IL; Diablo Valley Oncology Hematology Medical Group, Pleasant Hill, CA; Phoenix Baptist Hospital, Phoenix, AZ; Porto, Senhora da Hora, Portugal; Tower Outpatient Surgery Center, Los Angeles, CA and University of South Florida, Tampa, FL.

Body: Objective

To assess the safety and efficacy of single-fraction, intra-operative radiation therapy (IORT) delivered as a boost using the Xofig[®] Axxent[®] Electronic Brachytherapy System[®] (eBx[®]) immediately following surgical resection for treatment of early stage breast cancer.

Methods

This phase 4, open-label, single-arm, prospective, non-randomized trial is still enrolling participants and is currently being conducted at 26 hospitals in the USA (25) and Portugal (1). 878 participants with biopsy-proven ductal carcinoma in situ (DCIS) or invasive ductal carcinoma who met the inclusion criteria underwent lumpectomy followed by single-fraction IORT to the lumpectomy cavity. Briefly, a small, presterilized lead shield piece was placed on the chest wall to reduce the dose to the ribs, and then a balloon applicator, suitable to the surgical bed, was placed in the lumpectomy cavity and inflated with saline (30-75 cc); skin was temporarily closed over the balloon and ultrasound was used to confirm a balloon surface-to-skin distance ≥ 1.0 cm. The Xofig System was used to deliver the 20 Gy dose at the balloon applicator surface. The balloon was deflated, lead shield and balloon removed and the surgical site sutured. Upon the presence of additional risk factors, 37 participants subsequently received whole breast radiation therapy (WBRT); thus, these participants received an unplanned IORT boost and were removed from the primary analysis but will continue to be followed for the duration of the 10-year study. Cosmesis (Harvard Scale) was assessed in this subset of participants. The primary outcome for the main trial is recurrence of ipsilateral breast tumor at 5 years. Trial Registry: ClinicalTrials.gov; Identifier: NCT01644669.

Early Findings

37 boost participants received WBRT (up to 50 Gy) after IORT (36 received the prescribed 20 Gy dose; one received 14 Gy). Mean follow-up time was 430 days (range 13-1119). Mean age at IORT was 62 years (range 45-78). Boost participants had either DCIS (N=5) or invasive ductal carcinoma (N=32). The DCIS nuclear grade was high (N=3), intermediate (N=1), or low (N=1). Invasive cancers were Grade 1 (N=15), 2 (N=10), 3 (N=6), or unknown (N=1). 29 participants had T1, 3 had T2, and 5 had Tis lesions. Mean tumor size was 13.04 ± 10.26 mm. For the two participants who have reached 3-year follow-up, cosmesis was excellent (N=1) and fair (N=1). For the six participants who have reached 2-year follow-up, cosmesis was excellent (N=4), good (N=1), and fair (N=1). There was one serious adverse event with a Grade 3 for skin necrosis. The most frequent side effects were seroma (10%), edema (9%), pain (9%), erythema (6%), and induration (5%). There have been no deaths, recurrences, or new primary tumors among the boost participants to date.

Conclusions

Early results from this multi-center trial suggest that IORT as a tumor-bed boost using the Xofig Axxent eBx System at the time of breast conservation surgery is safe and has low morbidity. To date, the majority of participants receiving an unplanned IORT boost have had excellent to good cosmetic results and the majority of adverse events have been low-grade.

Funding

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2016 San Antonio Breast Cancer Symposium

Publication Number: P1-10-21

Title: Influence of timing of radiation therapy following breast-conserving surgery on 10-year disease-free survival

van Maaren MC C, Bretveld RW W, Jobsen JJ J, Veenstra R, Groothuis-Oudshoorn KCGM CGM, Struikmans H, Maduro JH H, Strobbe LJA JA, Poortmans P and Siesling S. Netherlands Comprehensive Cancer Organisation, Utrecht, Netherlands; Medical Spectrum Twente, Enschede, Netherlands; Dutch Institute for Clinical Auditing, Netherlands; MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, Netherlands; Medical Center Haaglanden, The Hague, Netherlands; Leiden University Medical Center, Leiden, Netherlands; University Medical Center Groningen, University of Groningen, Groningen, Netherlands; Canisius Wilhelmina Hospital, Nijmegen, Netherlands and Radboud university medical center, Nijmegen, Netherlands.

Body: Background

In the Netherlands, one of the indicators of quality of care is that radiation therapy (RT) should start within six weeks following breast-conserving surgery (BCS). However, there is still much controversy regarding timing of RT in literature. This study investigated the effect of timing of RT on disease-free survival (DFS) in a Dutch nationwide population-based cohort.

Methods

All women diagnosed with primary invasive stage I-IIIa breast cancer in 2003, treated with BCS plus RT, of whom the start date of RT was known, were included. Patients who received chemotherapy between surgery and RT were excluded, as this affects delay. Patients were categorised into three groups: <42 days, 42-55 days and >55 days, between surgery and start of RT. The primary outcome was 10-year DFS. Secondary outcomes were 10-year locoregional recurrence-free (LRRFS) and distant metastasis-free survival (DMFS). Multivariable Cox regression was used to correct for confounding. Since adjuvant systemic therapy largely influences DFS, all analyses were stratified for use of adjuvant systemic therapy (chemotherapy and/or endocrine therapy).

Results

In total, 2,759 patients were included. The median number of days between BCS and RT was 45 (IQR 37-54 days). The hazard ratio (HR) for 10-year DFS was 0.79 (95% CI: 0.65-0.96) for 42-55 days and 0.71 (95% CI: 0.56-0.90) for >55 days, both compared to <42 days. While no significant differences in 10-year LRRFS were found, 10-year DMFS (HR 0.64 [95% CI: 0.45-0.91]) was significantly higher for BCS-RT interval >55 days compared to <42 days. After stratification, no significant differences were found for any outcome in patients not treated with adjuvant systemic therapy, while in patients who were treated with adjuvant systemic therapy, 10-year DFS was significantly improved for 42-55 days (HR 0.70 (95% CI: 0.51-0.97) and >55 days (HR 0.63 (95% CI: 0.42-0.96) compared to <42 days. Significantly improved 10-year DMFS was confirmed after stratification for longer delays (HR 0.69 (95% CI: 0.47-1.00 for 42-55 days) and 0.59 (95% CI: 0.36-0.96) for >55 days, compared to <42 days)

10-year DFS, LRRFS and DMFS for different time intervals between BCS and RT, stratified for use of adjuvant systemic therapy

	Entire cohort (n=2,759)	No adjuvant treatment (n=1,761)	Adjuvant treatment (n=998)
Time interval	HR [95% CI]*	HR [95% CI]*	HR [95% CI]*
10-year DFS			
<42 days	1	1	1
42-55 days	0.79 [0.65-0.96]	0.83 [0.65-1.05]	0.70 [0.51-0.97]
>55 days	0.71 [0.56-0.90]	0.77 [0.57-1.03]	0.63 [0.42-0.96]
10-year LRRFS			
<42 days	1	1	1
42-55 days	0.74 [0.51-1.06]	0.79 [0.52-1.21]	0.55 [0.28-1.11]
>55 days	0.90 [0.59-1.37]	0.99 [0.61-1.60]	0.67 [0.29-1.57]
10-year DMFS			
<42 days	1	1	1

42-55 days	0.90 [0.69-1.17]	1.13 [0.77-1.66]	0.69 [0.47-1.00]
>55 days	0.64 [0.45-0.91]	0.73 [0.44-1.22]	0.59 [0.36-0.96]

* Corrected for all relevant confounders. HRs in bold are statistically significant ($p < 0.05$)

Conclusion and interpretation

In conclusion, patients treated with adjuvant systemic therapy following RT showed increasing 10-year DFS and DMFS with longer BCS-RT intervals, which was not the case for patients not receiving adjuvant systemic therapy. Possible explanations for these results have to be explored further.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-01

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-02

Title: A comparison of models (physician, the Van Nuys prognostic index, the Memorial-Sloan-Kettering Cancer Center DCIS nomogram) to predict ipsilateral breast events in patients with ductal carcinoma in situ (DCIS) of the breast after breast-conserving surgery failed to replicate results of the oncotype DCIS recurrence score

Leonard C, Lei R, Antell A, Nowels M, Fryman S, Howell K and Dennis C. Rocky Mountain Cancer Centers, Littleton, CO; Rocky Mountain Cancer Centers, Boulder, CO; University of Colorado Denver, Denver, CO and Rocky Mountain Cancer Centers, Aurora, CO.

Body: Purpose: To compare ipsilateral breast event (IBE) risks in patients with DCIS of the breast post-lumpectomy, as estimated by breast radiation oncologists, the Van Nuys Prognostic Index (VNPI) studies, the Memorial Sloan Kettering Cancer Center (MSK) DCIS nomogram, and the 12-gene DCIS Recurrence Score assay.

Materials/Methods: 12-gene DCIS Recurrence Score of 91 DCIS lumpectomy cases were identified from our practice. Clinicopathologic factors (excluding DCIS score) were summarized for blinded review. 3 radiation oncologists independently estimated the 10-year IBE risk and rated the impact of age/menopausal status, tumor morphology, tumor span, and margin width. Corresponding VNPI and MSK nomogram estimates were generated. Differences and correlations between the IBE estimates and clinicopathologic factors were evaluated with univariate and multivariate analysis.

Results: Median age was 60, 25% margins < 3 mm, 48% DCIS span > 10 mm, 48% would have been ineligible for E5194. All comparisons were initially examined for similarity (non-significant differences) and subsequently tested for a significant correlation. In three possible physician comparisons only 1 (AA-CL) had similar IBE estimates and strong correlation ($r=0.792$). Comparison of physicians to either VNPI nomogram or MSK DCIS calculator had similar estimates with strong correlation in only 2 of 6 possible comparisons in the all cases and the E5194 eligible patient cohorts respectively; and in 1 of 6 comparisons in the E5194 ineligible cohort. The DCIS Recurrence Score showed no strong correlation in regard to similar IBE estimates for any comparisons including either physicians, VNPI nomogram or MSK DCIS calculator. Tumor size was highly correlated with all physician IBE estimates ($r = 0.547$ to 0.799), while margin size showed moderate to high inverse correlations ($r = -0.324$ to -0.619). All 3 physicians rated margin width as having the most impact on their IBE risk estimates, then tumor morphology and tumor span.

Source of Estimate	All Cases (n = 91)	E5194 Eligible (n = 47)	E5194 Ineligible (n = 44)
DCIS Score	15.1%, 5.3 (14.0-16.2)	14.2%, 4.8 (12.8-15.6)	16.1%, 5.7 (14.3-17.8)
MSK DCIS Nomogram (Rudloff et al., 2010)	18.8%, 7.7 (17.2-20.4) 23.4%, 18.6 (19.6-27.3)	16.1%, 4.1 (14.9-17.3)	21.7%, 9.4 (18.8-24.6)
VNPI studies	23.4%, 18.6 (19.6-27.3)	15.6%, 15.5 (11.0-20.1)	31.9%, 18.1 (26.4-37.4)
AA	18.9%, 14.1 (16.0-21.9)	11.7%, 8.8 (9.1-14.3)	26.6%, 14.7 (22.2-31.1)
CL	19.9%, 11.8 (17.5-22.4)	14.5%, 6.6 (12.5-16.4)	25.8%, 13.3 (21.7-29.8)
DC	26.8%, 13.9 (23.9-29.7)	19.8%, 5.9 (18.1-21.6)	34.2%, 16.1 (29.3-39.1)

Conclusion: IBE risk estimates for this cohort of DCIS cases vary significantly among commonly available clinical predictive tools and individual physician estimates. Surgical margins and tumor size continue to factor prominently in physician decision algorithms. In our observations, neither the VNPI nomogram, MSK DCIS calculator nor physician IBE risk estimates have similar values with strong enough correlation to reliably replicate the DCIS Recurrence Score.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-03

Title: Rates of ipsilateral breast tumor recurrence (IBTR) following breast conserving surgery (BCS) and hypofractionated radiotherapy for ductal carcinoma in situ (DCIS)

Dumitru D, Benson J, Wishart G and Provenzano E. Addenbrooke's Hospital; Cambridge Breast Unit, Cambridge, Cambridgeshire, United Kingdom and Anglia Ruskin University, Cambridge, Cambridgeshire, United Kingdom.

Body: Background: The risk of IBTR following BCS for DCIS is dependent on both tumor and treatment-related factors including surgical margin width and adjuvant therapies. Management strategies should be risk stratified to avoid over/under-treatment with radiotherapy and endocrine therapy. De-escalation of treatments with safe omission of adjuvant therapies may demand a minimum margin of surgical clearance for non-high grade and selected high grade lesions.

Methods: A retrospective analysis was undertaken to examine rates of IBTR among patients undergoing BCS for core biopsy-proven DCIS between 1999 and 2010 when a minimum margin width of 5mm prevailed. The local institutional database identified 1260 DCIS cases with or without an invasive component among whom 323 had pure DCIS diagnosed mainly on screening (>90%). A total of 176 patients were treated with BCS alone (27.5%) or combined with breast radiotherapy [15 fractions of 2.67Gy to total dose of 40Gy without boost]. No patients received any form of hormonal therapy (tamoxifen/aromatase inhibitor). Ten patients died from non-breast cancer causes prior to development of IBTR leaving 167 unilateral and 1 bilateral patient (i.e. 168 cases) for analysis with high (72%), intermediate (17.8%) and low (9%) grade DCIS (or ungradeable).

Results: At a median follow up of 126 months (range 46 – 180) a total of 14 patients have developed IBTR as a first event (8.33%). Approximately half of these were non-invasive (n= 8) and half invasive (n=6). Half of DCIS recurrences (4/8) occurred in the first 12 months following surgery and all recurrent DCIS cases were manifest by 3 years compared with a steady recurrence of invasive disease up to 10 years of follow up. One case of invasive disease died from subsequent distant metastases with visceral deposits. There was no significant difference in rates of recurrence with (9/121) or without (5/46) irradiation ($p=0.534$). Among the 14 recurrent cases, 12 had conformal radial margins of 5mm whilst 2 cases had single minimum margins of 3mm and 2mm (accepted without re-excision due to advanced age, co-morbidity or lesion at edge of breast tissue). Characterization of molecular profiles (ER, HER2, Ki-67) for recurrent cases is ongoing.

Conclusion: These rates of local control with a target margin of 5mm and selective hypofractionated breast radiotherapy are consistent with published IBTR rates of approximately 1% per annum for DCIS patients treated with BCS and radiotherapy. Routine inclusion of hormonal therapy may be unnecessary for many patients receiving adjuvant radiotherapy with comparable 10 year recurrence rates of 7 - 8% reported in the International Breast Intervention Study (IBIS)-II.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-04

Title: Looking beyond the margins: Economic costs and complications associated with repeated breast-conserving surgeries

Metcalf LN N, Zysk AM M, Underwood HR R, Edelman G, Vu L, Cittadine AJ J, Hyer KB B and Thompson AM M. Health Care Services Corporation, Richardson, TX; Diagnostic Photonics, Inc., Chicago, IL and University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Although considerable attention has been drawn to the problem of repeat breast-conserving surgery (BCS), the costs and complications due to these additional operations are not well-characterized. In this work, a retrospective review of insurance claims data for BCS patients was performed to assess complications and economic outcomes.

Methods: Private claims data were analyzed for 9,837 women undergoing BCS for breast carcinoma between January 2010 and December 2013. Patients enrolled in insurance plans in IL, TX, NM, and OK were included. Patients undergoing a second open breast surgery (mastectomy or BCS) within 90 days of the initial BCS were classified as having a repeat surgery. Complications were identified via a set of 8 CPT and 25 ICD9 diagnosis and procedure codes related to breast cancer treatment. The analysis included these complications and the total cost of all allowed healthcare claims within two years following diagnosis.

Results: 7,555/9,837 patients (77% \pm 0.8%, 95% confidence interval) had one BCS operation, and 2,282 patients (23% \pm 0.8%) had at least one repeat surgery. The mean patient age was 53 years. Women who underwent an additional operation waited an average of 24 days for the procedure.

The mean two-year total costs for patients undergoing a single BCS was \$89,016 (\pm \$1,884), and the cost for patients undergoing a repeat breast surgery was \$105,088 (\pm \$3,680; $p < 0.0001$), \$100,637 (\pm \$4,219) for a second BCS and \$115,292 (\pm \$7,259) for subsequent mastectomy. The mean added cost due to a repeat surgery was \$16,072.

The percentage of patients experiencing at least one complication was 23.6% (\pm 1.0%) for those undergoing one BCS only and 34.8% (\pm 2.0%) for those undergoing a repeat operation ($p < 0.0001$). Patients undergoing repeated surgery were 88% more likely to experience multiple complications (5.5% \pm 0.5% vs. 10.4% \pm 1.3%) and nearly three times as likely to experience fat necrosis (2.5% \pm 0.4% vs. 7.2% \pm 1.1%). Infection, hematoma/seroma, and breast pain were the most common complications for patients who did not undergo a repeated surgery (9.9% \pm 0.7%, 8.7% \pm 0.6%, 6.9% \pm 0.6%). For patients undergoing a repeated surgery, infection, hematoma/seroma, and fat necrosis were the most common complications (15.3% \pm 1.5%, 13.9% \pm 1.4%, 7.2% \pm 1.1%).

The impact of repeated breast-conserving surgeries

	BCS, No Repeat	Repeat BCS	Convert to Mastectomy
Patients	76.8% (7,555)	16.2% (1,589)	7.0% (693)
Mean Two-Year Cost Per Patient	\$89,016	\$100,637	\$115,292
Patients with any Complication(s)	23.6% (1,783)	32.5% (516)	40.3% (279)
Patients with Infection	9.9% (746)	14.0% (222)	18.3% (127)
Patients with Hematoma/Seroma	8.7% (655)	12.8% (203)	16.6% (115)
Patients with Breast Pain	6.9% (525)	7.0% (111)	6.1% (42)
Patients with Fat Necrosis	2.5% (187)	7.6% (120)	6.5% (45)

Conclusions: For the 23% of women undergoing a second operation after BCS, complications were 48% more common, and the mean total cost of surgery was \$16,072 more, demonstrating statistically-significant evidence of a patient-centered and fiscal imperative to reduce reoperations in BCS for breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-05

Title: Influence of race and age on mastectomy rates in women with stage I, hormone-sensitive breast cancers: A SEER-based study

Bazan JG G, Bittoni MA A, Fisher JL L and White JR R. The Ohio State University, Columbus, OH.

Body: Background: Breast conserving therapy (lumpectomy [L] and breast radiotherapy [RT]) results in equivalent cancer control outcomes in comparison to mastectomy (M) for early stage breast cancer (BC) based on randomized controlled trials (RCT). Since 2004, RCT support that L alone without RT yields equivalent survival and acceptable local regional outcomes in women ≥ 70 years old with stage I (T1N0) hormone-sensitive (HS) BC on endocrine therapy. Based on this, we hypothesized that M rates should decrease substantially in this low risk elderly population and sought to examine the influence of race on M rates in this group and how these trends compare to younger aged Stage I HS patients.

Methods: We used the Surveillance Epidemiology and End Results (SEER) registry data to conduct this study. We included women with T1N0 HS BC classified as either ER-positive or PR-positive from 2000-2012 divided into 2 age groups [elderly (≥ 70 years old) and non-elderly (20-69 years old)] and 3 race groups [white, black, and Asian-Pacific-Islander/American Indian/Alaskan Native (API)]. We compared M rates in women diagnosed before 2004 compared to those diagnosed from 2005-2012. Statistical analyses were performed using differences in proportions ($p < 0.05$ considered statistically significant).

Results: 261,079 women met the study criteria (N=87,009 elderly; N=174,070 non-elderly). In elderly Stage I HS BC, a 5.2% reduction in the M rate is seen: 32.6% before 2004 to 27.4% after 2004 ($p < 0.0001$). M rates remained higher (with less reduction) in elderly Black (30.8 %) and API (33.6 %) vs. White (26.8%) [$p < 0.0001$ for White vs. Black and for White vs. API]. In non-elderly Stage I HS BC, after 2004 M rates increased from 29.2% to 31.8% ($p < 0.0001$). Non-elderly white women had the largest absolute increase in M rates (31.2% vs. 28.5%, $p < 0.0001$) followed by API women (35.1% vs. 37.1%, $p = 0.0222$). M rates did not change after 2004 in non-elderly black women (31.7% vs. 31.7%, $p = 0.9953$).

Conclusions: In patients with favorable stage I HS BC, M rates have decreased only modestly in elderly women since 2004 when L alone w/o RT was established as appropriate treatment. In comparison, M has increased since 2004 in non-elderly women. These trends are driven mostly by white women in both the elderly and non-elderly. Further research is needed to identify why M, which is associated with higher cost and morbidity than L alone, has not changed substantially in elderly very favorable BC, particularly for non-whites.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-06

Title: Learning curve for the SAVI SCOUT breast localization and surgical guidance system

Shukla SC C, Shivers SC C, Mattingly A, Russell S, Mehindru A, Carter E and Cox CE E. Morsani College of Medicine at the University of South Florida (USF), Tampa, FL.

Body: Background: The gold standard for localizing non-palpable breast lesions for surgical excision is wire localization (WL). Multiple disadvantages for WL include complicated scheduling and migration of the wire after placement. Radioactive seed localization (RSL) mitigates these disadvantages, but regulatory requirements regarding radiation limit more universal adoption. The SAVI SCOUT surgical guidance system (an FDA cleared medical device) eliminates the drawbacks of WL without the regulatory requirements of RSL. SCOUT utilizes electromagnetic wave technology and infrared light to provide intra-operative guidance during surgical excision. The purpose of this study is to describe the learning curve associated with adoption of this new technology.

Method: An IRB-approved prospective, single-arm, multi-site trial enrolled women with non-palpable breast lesions requiring localized surgical excision. After informed consent, a radiologist or surgeon used imaging guidance to implant the SCOUT reflector into the target lesion. Intraoperatively, the surgeon used SCOUT for localization of the reflector and removal of the target lesion. We evaluated the association of several independent variables with respect to successful localization and surgical excision including: tumor side, tumor quadrant, distance of reflector from the skin, and the number of SCOUT localized breast excisions performed by operating surgeon up to the 1st five cases. We studied the relationship between these independent variables and the following dependent variables: reflector detection post-placement, reflector detection pre-incision, and reflector localization post-incision. Statistical analysis utilized the z-test to perform a two-sided test of equality at an alpha level of 0.05 with adjustment for multiple comparisons by the Bonferroni method. T-tests were used to perform two-sided tests of equality for numeric variables.

Results: Across 11 institutions, 16 surgeons performed a total of 153 surgical excisions. Overall success rates of reflector detection pre-incision and post-incision were 98% (150/153) and 99% (151/153), respectively. The reflectors were successfully removed in 100% (153/153) of cases. Difficulty with reflector detection immediately post placement was significantly associated with reflectors more than 4 cm ($P=0.034$) or 5 cm ($P=0.007$) from the skin, or the procedure being the 1st SCOUT case by the operating surgeon ($P=0.036$). Operating surgeons performing their 1st SAVI localization procedure were significantly associated with difficult reflector detection post-incision ($p=0.044$). Subsequent procedures, up to the first five SCOUT localizations, noted no significant difficulty with reflector detection.

Conclusions: The SAVI SCOUT surgical guidance system is a viable surgical localization procedure for non-palpable breast lesions. Surgeons were 100% successful at removing the reflectors during surgical excision. Difficulty with reflector detection was not noted after the surgeon's 1st SCOUT procedure. Overall, it appears the learning curve for reflector placement and localization for non-palpable breast lesions is relatively short. However, depth of the reflector in relation to skin likely affects reflector detection during this early learning period.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-07

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-08

Title: Tumor and procedural factors associated with positive margins at lumpectomy in women undergoing breast conservation surgery

Boughey JC C, Keeney MG G, Glasgow AE E, Keeney GL L and Habermann EB B. Mayo Clinic, Rochester, MN.

Body: Background:

Negative margins are important in decreasing risk of local recurrence after breast conservation surgery. Further, positive margins on final pathology require a second operation, burdening patients and increasing costs. We identified factors predicting positive margins at lumpectomy prompting intraoperative re-excision in a large referral center.

Methods

With IRB approval we reviewed all breast cancer lumpectomy cases from January 2012 to December 2013. Associations between rates of positive margin (defined as tumor at ink) and patient and tumor factors were assessed using chi square tests and univariate and adjusted multivariable logistic regression, overall and stratified by DCIS or invasive cancer.

Results

385 patients (105 DCIS and 280 invasive disease) were identified. Overall positive margin rate at lumpectomy requiring intraoperative re-excision was 62.3% and was higher in DCIS than in invasive disease (78.1% vs 56.4%, OR=2.78, p=0.001). Positive margin rates did not vary by surgeon, patient age, ER, PR or HER2 status of the tumor. On univariate analysis higher tumor grade was associated with a higher margin positive rate (grade 3 vs grade 1, OR=1.71, p=0.049).

Within the 105 DCIS cases, no factors had statistically significantly different odds of positive margins on univariate or multivariable analysis.

Within the 280 cases of invasive breast cancer, factors independently associated with lower odds of margin positivity were neoadjuvant chemotherapy (OR 0.30, p=0.037, relative to no neoadjuvant therapy) and seed localized excision (OR 0.24, p=0.03, relative to no localization).

Multivariable Logistic Regression* Modeling

Variable	DCIS		Invasive Disease	
	Odds Ratio	P-value	Odds Ratio	P-value
Age 50+ vs Age <50	2.86	0.11	1.59	0.23
Grade 2 vs Grade 1	0.94	0.92	1.50	0.21
Grade 3 vs Grade 1	3.25	0.12	1.58	0.37
Estrogen Receptor Positive vs Negative	3.95	0.22	1.64	0.42
Progesterone Receptor Positive vs Negative	0.84	0.87	1.11	0.84
Her2 Positive vs Negative			1.27	0.68
Stage T2 vs T1			2.54	0.45
Stage T3 vs T1			2.18	0.69
Ki67 <15% vs 15%+			0.57	0.09
Neoadjuvant Chemotherapy vs None			0.30	0.04
Seed Localization vs No Localization			0.24	0.03
Wire Localization vs No Localization			0.33	0.13
Intraoperative Ultrasound vs No Localization			0.67	0.53

*adjusted for individual surgeons (all p >0.05)

Conclusion

DCIS was associated with higher rate of positive margins at lumpectomy than invasive breast cancer. Within invasive disease, neoadjuvant chemotherapy and seed localization were associated with lower rates of margin positivity. Strategies to decrease positive margins would be best employed in cases of lumpectomy for DCIS and for invasive disease treated without neoadjuvant chemotherapy. Seed localization may be one strategy to lower positive margin rates.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-09

Title: The impact of a change in margin width on rates of re-excision following breast conserving surgery

Jiwa NE E, Ayyar S, Provenzano E and Benson JR R. Addenbrookes Hospital, Cambridge, United Kingdom.

Body: Background: Surgical margin status is a predictor of ipsilateral breast tumor recurrence but the definition of an adequate surgical margin following breast conserving surgery (BCS) remains controversial despite published recommendations for acceptance of 'no tumor at ink'. BCS can be compromised by the need for a re-excision procedure which incurs inconvenience to patients and additional healthcare costs. We have previously shown that reducing the margin mandate from 5mm to 2mm does not influence rates of re-excision and the impact of adopting a more stringent margin policy of 'no tumor at ink' is now reported.

Methods: A retrospective analysis examined rates of re-excision amongst patients undergoing BCS for both invasive carcinoma with or without admixed ductal carcinoma in situ (DCIS) or pure DCIS over two sequential 12 month time periods either prior to (GROUP 1; n=225) or immediately following (GROUP 2; n=238) a change in minimum margin policy from 2mm to 'no tumor at ink' when the latter was deemed an adequate definition of a negative margin. A total of 611 patients underwent non-reconstructive breast surgery for symptomatic and screen-detected cancers during these time periods among whom 463 received BCS. Wire-localization was undertaken in 51% (114/225) and 42% (101/238) of patients in groups 1 and 2 respectively. Data was extracted from electronic patient records of histopathology reports and clinic letters with further information available from documentation of MDT discussions. Statistical analysis used Fisher-Exact test.

Results: Rates of re-excision were significantly lower for group 2 (32/238=13%) compared with group 1 (48/225=21%). Re-excision encompassed cavity re-excision alone, cavity re-excision followed by mastectomy and completion mastectomy. Residual disease in re-excision specimens was significantly higher for group 2 (40.6%) compared with group 1 (16.6%) [p=0.02] with more than one-third of cases of residual disease in group 2 being exclusively DCIS. Three patients in each group required 2 additional operations to achieve negative margins. For groups 1 and 2, three-quarters or more cases (87% and 77% respectively) had only 1 or two margins positive and 2 cases in group 2 were re-excised for reasons other than a positive margin (e.g. tumor type). For group 1, re-excision cases were prompted by margin categories of 0mm (n=22), <1mm (n=18) and ≥1mm; <2mm (n=8) whilst for group 2 all re-excisions were prompted by tumor at ink (0mm). A hypothetical minimum margin of 1mm would have reduced the re-excision rate for group 1 to 40/225 or 17.7%.

Conclusion: Re-excision was usually prompted by margins of <2mm when a 5mm margin policy prevailed. A more relaxed margin mandate of 'no tumor at ink' has led to halving of re-excision rates from 21% to 13% as opposed to 17.7% for a minimum margin of 1mm. This reduction in rates of re-excision is accompanied by an increase in the proportion of cases with residual disease in re-operation specimens. Longer-term follow up is essential to monitor in-breast local recurrence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-10

Title: Clinical applications of near infra-red imaging system for localization of non-palpable breast lesions in breast conserving surgery

Jung S-Y, Lee S and Kim SK. National Cancer Center, Goyang, Korea.

Body: Backgrounds: Localization of non-palpable breast lesions is important for obtaining tumor-free resection margin and achieving better cosmetic outcome. Near infra-red (NIR) imaging system has been introduced for localization in breast surgery. This study aimed to evaluate the feasibility of localization using NIR imaging system in breast conserving surgery (BCS) (ClinicalTrials.gov identifier: NCT 02172989/ NCT02473159).

Materials and Methods: Between June 2014 and October 2015, 20 women with benign neoplasm and 5 patients with early breast cancer were enrolled and underwent BCS using NIR imaging system. Before surgery, Indocyanine green was injected intratumorally in benign lesions and peritumorally for resection margins in early breast cancer. Their pathologic results for resection volume and re-excision rate were compared with those of the patients with 99 benign neoplasm and 203 early breast cancer who were treated with BCS by conventional method, respectively.

Results: In the patients with benign lesions, the mean size in sonography was 1.48 ± 0.98 cm in NIR group and 1.32 ± 0.96 cm in conventional group ($P=0.4$). There was no difference of pathologic lesion size and excised specimen size between these groups (1.57 ± 0.78 cm vs 1.42 ± 0.80 cm in pathologic lesion size; $P=0.63$, 4.28 ± 0.48 cm vs 4.15 ± 1.37 cm in specimen size; $P=0.73$). In NIR group, the ratio of excised specimen/lesion was lesser than that of conventional group (3.19 ± 1.40 vs 4.31 ± 3.86 ; $P=0.008$). In addition, positive margin rate after BCS in early breast cancer patients was 0% in NIR group and 19.7% in conventional group. And re-excision rate was 0% and 4.4%, respectively.

Conclusions: This study showed that localization using NIR image system could be a feasible method to obtain safe resection margins and optimum resection volumes in patients undergoing breast conserving surgery.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-11

Title: Evaluating cosmetic outcome following breast conserving surgery in trials; panel versus objective evaluation and the role of patient reported outcome measures (PROMs)

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Body: Objective

Cosmetic outcome is an important quality of life related endpoint following breast conserving surgery (BCS). We aim to compare the cosmetic outcome evaluated by panel and objective evaluation (BCCT.core software). Second patient reported outcome measures (PROMs) are compared to cosmetic evaluation by panel and BCCT.core.

Methods

Sixty-eight breast cancer patients were included after breast conserving surgery between 2007-2012. Cosmetic outcome was evaluated by; two independent 6-member panels, the BCCT.core by two observers and PROMs (EORTC-QLQ-C30/BR23, EQ-5D-5L and BREAST-Q 'breast conserving module'). First, reproducibility, repeatability and overall agreement of panel and BCCT.core was analysed using the interclass correlation coefficient (ICC). Second, the correlation between panel/BCCT.core with PROMs was analysed using the Spearman's rank correlation coefficient (spearman's ρ). Sensitivity of all PROMs to differentiate between a 'good' or 'bad' cosmetic outcome was evaluated.

Results

Sixty-four patients (94.1%) completed the EORTC-QLQ-C30/B23, 58 (85.3%) the EQ-5D-5L and BREAST-Q. Repeatability between both panels and BCCT.core observers was respectively 0.93 and 0.86 (ICC). Reproducibility for panel 1 and BCCT.core 1 was respectively 0.93 and 0.96. Overall agreement between panel and BCCT.core ranged between 0.59 – 0.69.

Table 1. Inter- and intra-observer agreement for cosmetic outcome - Interclass Correlation Coefficient (95% CI)

	Panel 1	Panel 2	BCCT.core 1	BCCT.core 2
Panel 1	0.99 (0.98-0.99)	0.94 (0.90-0.96)	0.69 (0.54-0.79)	0.61 (0.43-0.73)
Panel 2			0.66 (0.5-0.77)	0.59 (0.42-0.72)
BCCT.core 1			0.93 (0.84-0.97)	0.85 (0.77-0.91)
BCCT.core 2				

For PROMs the BREAST-Q showed the strongest correlation with panel and BCCT.core, 0.32-0.47 spearman's ρ . The BREAST-Q significantly differentiated between a 'good' or 'bad' cosmetic outcome based on panel and BCCT.core ($p < 0.05$)

Table 2 PROMs according to a 'good' or 'bad' cosmetic outcome

	Panel 1		p-value	BCCT. core 1		p-value
	Good - median (IQR)	Bad median (IQR)		Good - median (IQR)	Bad - median (IQR)	
EORTC-QLQ-C30; Global Health Status*	83.3 (75-100)	83.3 (66.7-100)	0.92	83.3 (75-100)	83.3 (66.7-100)	0.82
EORTC-QLQ-B23; Body image*	95.8 (72.9-100)	75 (50-100)	0.15	95.8 (68.8-100)	83.3 (66.7-100)	0.31
EQ-5D-5L; Health score*	80 (68.5-91)	90 (72.5-95)	0.19	80 (71.3-90)	90 (65-95)	0.38
EQ-5D-5L; Index value*	0.87 (0.76-1)	0.84 (0.82-1)	1	0.85 (0.74-1)	0.843 (0.811-1)	0.81
BREAST-Q; satisfaction with breast*	68.5 (56.5-90)	52 (41.5-67)	0.02	68.5 (57.5-89.5)	52 (41-68)	0.03

IQR = Interquartile range *Higher scores represent higher quality of life

Conclusion

Comparable good reproducibility and repeatability was found for panel and BCCT. PROMs showed limited agreement but the PROM BREAST-Q was able to differentiate between a good or bad cosmetic evaluation. Combining PROMs with panel *or* BCCT.core in future trials evaluating cosmetic outcome following BCS could further improve and evaluate the use of PROMs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-12

Title: Re-evaluating outcomes of partial-breast irradiation using multicatheter brachytherapy for Japanese patients with breast cancer by European brachytherapy phase 3 trial

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Body: [Purpose] The Groupe European de Curietherapie-European Society for Therapeutic Radiology and Oncology (GEC-ESTRO) has reported the equivalent outcomes of partial-breast irradiation (PBI) using multicatheter interstitial brachytherapy (MCB) to whole-breast irradiation (WBI) in breast-conserving therapy, showing that the incidence of local recurrence was 1.44% with MCB-PBI and 0.92% with WBI. Based on the trial, MCB-PBI could be considered as an alternative method to WBI for low-risk breast cancer patients. However, it would be difficult to translate it directly into Japanese community practice. After categorization of Japanese patients using the inclusion and exclusion criteria of this trial, our databases were re-evaluated to validate the data for Japanese patients, and the possibility to extend the candidate for MCB-PBI was also investigated.

[Methods] Patients undergoing BCT were retrospectively examined between November 2007 and December 2015. The technique involved an open-cavity implant with a dose of 32 Gy in eight fractions. WBI was performed with a total dose of 50 Gy in fractions of 2 Gy. The 4-year clinical outcomes of MCB-PBI were evaluated in the two distinct categories, and comparisons of outcomes between MCB-PBI and WBI were performed in patients with unfavorable features.

[Results] Of a total of 501 lesions undergoing BCT, 301 lesions were treated with MCB-PBI and 200 lesions with WBI. At a median follow-up time of 52 months, the 4-year rate of ipsilateral breast tumor recurrence (IBTR)-free, disease-free, and overall survival in patients with MCB-PBI and WBI were 98.9% vs. 98.0% ($p = 0.56$), 97.0% vs. 95.3% ($p = 0.78$), and 99.6% vs. 98.2% ($p = 0.38$), respectively. In the exclusion cohort treated with MCB-PBI, IBTR-free and disease-free survival were significantly poorer than in the inclusion cohort. However, no significant differences in the outcomes between the two radiotherapy techniques were demonstrated with respect to either IBTR-FS (95.0% vs. 97.2%, $p = 0.24$), DFS (95.0% vs. 95.8%, $p = 0.31$), or OS (100% vs. 99.0%, $p = 0.80$) in patients with exclusion criteria

Univariate and multivariate analysis of prognostic factors for IBTR and breast cancer event

Variables	IBTR			Locoregional and distant recurrences		
	P-value			P-value		
	Univariate	Multivariate	HR (95% CI)	Univariate	Multivariate	HR (95% CI)
Age \geq 40 vs < 40years	.25	—	2.49 (0.53–11.72)		—	0.90 (0.21–3.85)
Axillary node negative vs. positive	<.05	<.05	4.09 (1.18–14.12)	<.05	<.05	2.75 (1.19–6.36)
Margin negative vs. positive	<.01	<.01	5.58 (1.54–20.29)	.075	.068	2.55 (0.93–6.97)
WBI vs. MCB-PBI	.56	—	0.69 (0.20–2.39)	.78	—	0.89 (0.39–2.03)

[Conclusions] To our knowledge, this study includes the largest cohort of Asian patients undergoing MCB-PBI. Although the retrospective chart review with relatively short follow-up time and small number of patients does not allow reaching any definite conclusion, we could expect the same outcomes by MCB-PBI as those in the GEC-ESTRO trial with respect to the tumor control

only for Japanese but for Asian patients with breast cancer. Moreover, no negative impact on the outcomes of MCB-PBI as compared to WBI was not found in patients with exclusion criteria.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-13

Title: Re-excision rate in breast conservation surgery after neoadjuvant chemotherapy

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Body: Background : The goal of neoadjuvant chemotherapy is to reduce tumor size and convert mastectomy to breast-conservation surgery (BCS). In response to neoadjuvant chemotherapy, the regression rate and pattern of breast cancer is variable. And the re-excision rate to secure negative resection margin is reported limitedly in this case. The purpose of this study was to compare the re-excision rate and BCS success rate of patients who received and who did not received neoadjuvant chemotherapy.

Method : In this retrospective cohort study, between January 2009 and December 2012, total 256 women were included who had clinical T2 breast cancer and were planed to receive BCS as initial operation or neoadjuvant chemotherapy. Fifty-nine patients of them received neoadjuvant chemotherapy. Clinical data were collected including age, preoperative or initial clinical tumor size, mammographic microcalcifications, ultrasound multifocality and axillary nodal status, retrospectively. In the resected specimen from BCS, we reviewed the pathologic tumor size, multifocality, histologic type, hormone receptor and Her-2-neu status, ki67, DCIS and EIC component. The re-excision rate and BCS success rate were investigated. Univariate analysis and regression model were used for identify clinicopathologic factors associated with re-excision. To reduce the effect of selection bias, propensity score matching-based anaylsis was also performed.

Results : Of the 256 patients, 178 patients (90.4%, 178/197) received BCS finally in neoadjuvant group and 56 patients (94.9%, 56/59) in non-neoadjuvant group ($p=0.406$). There was no statistical difference in the re-excision rate between two groups (35.6% (21/59) in neoadjuvant group vs 34.0% (67/197) in non-neoadjuvant group, $p=0.946$). In propensity-matched cohorts (N=118), the re-excision rate was same in two groups (35.6% (21/59) in neoadjuvant group vs 35.6% (21/59) in non-neoadjuvant group, $p=1.000$). BCS success rate was higher in neoadjuvant group(94.9% 56/59) than non-neoadjuvant group (86.4%(51/59)), but there was no statistical difference ($p=0.205$). In this cohorts, clinicopathologic factors associated with re-excision were pathologic multifocality (OR=4.56, $p=0.0142$), high ki67 ($\geq 50\%$) (OR=0.7, $p=0.0243$) and DCIS component (OR=2.67, $p=0.0261$) in logistic regression model.

Conclusion: This study showed neoadjuvant chemotherapy could increase the BCS success rate but could not decrease the re-excision rate. The re-excision rate is more associated with pathologic finding rather than effect of neoadjuvant chemotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-11-14

Title: Breast conserving therapy and mastectomy revisited: overall and breast cancer-specific survival and the influence of age, stage, receptor status and comorbidities in 143,376 patients

Legendijk M, van Maaren MC C, Saadatmand S, Strobbe LJA JA, Poortmans P, Koppert LB B, Tilanus-Linthorst MMA MA and Siesling S. Erasmus MC Cancer Institute, Rotterdam, Zuid-Holland, Netherlands; Netherlands Comprehensive Cancer Organisation, Utrecht, Netherlands; Maxima Medical Center, Velthoven, Brabant, Netherlands; Camisius Whilehelmina Hospital, Nijmegen, Netherlands and Radboud University Medical Center, Nijmegen, Netherlands.

Body: *Background*

Large randomised controlled trials confirmed in the 80's equal survival for breast-conserving surgery plus radiation therapy (BCT) and mastectomy. Recently, observational studies challenged this equivalence, showing superior survival rates for BCT. This study compared BCT with mastectomy on long-term overall (OS) and breast cancer-specific survival (BCSS) in a population-based cohort, investigating the influence of age, stage and presence of comorbidities.

Methods

Patients with primary Tis-2N0-2M0 stage breast cancer, diagnosed between 1999-2012, were selected from the Netherlands Cancer Registry. The cohort was divided into two time cohorts, 1999-2005 (to evaluate long-term outcome) and 2006-2012 (with modern adjuvant therapy). Cause of death was derived from the Statistics Netherlands (CBS). Multivariable analyses were performed separately for all invasive cancers, DCIS, T1-2N0-1 and T1-2N2 stages. In addition, the T1-2N0-1 stages were further stratified for age, receptor status and presence of comorbidities at diagnosis.

Results

A total of 143,376 patients were selected. In the 1999-2005 cohort, 51.4% of 65,666 patients were treated with BCT, compared to 58.9% of 80,772 patients in the 2006-2012 cohort.

In the 1999-2005 cohort, 9,413 patients (27.9%) died in the BCT group, compared to 14,956 patients (46.8%) in the mastectomy group. Of these deaths, 4,710 (50%) were breast cancer-related in the BCT group, compared to 7,845 (52.2%) in the mastectomy group. For all invasive carcinomas, BCT showed significantly higher adjusted BCSS [0.72 (95% CI: 0.69-0.76)] and OS [HR 0.74 (95% CI: 0.71-0.76)] than mastectomy. This remained significant in all other subgroups: DCIS and all T and N stages. Only for patients aged <40, BCT was equal to mastectomy regarding both BCSS and OS.

In the 2006-2012 cohort, 4,134 patients (8.7%) died in the BCT group, compared to 6,412 (19.3%) in the mastectomy group. Of these deaths, 2,036 (49.3%) were breast cancer-related in the BCT group, compared to 3,252 (50.7%) in the mastectomy group. Also for this cohort, BCT showed significantly higher adjusted BCSS [HR 0.75 (95% CI 0.70-0.80)] and OS [HR 0.67 (95% CI: 0.64-0.71)]. This remained significant for DCIS and T1-2N0-1M0 patients. Equal BCSS and OS for BCT and mastectomy was observed in patients with T1-2N2 stage breast cancer. Within the T1-2N0-1 subgroup, additional stratification for age, hormonal receptor status and presence of comorbidities showed significant superior BCSS and OS for BCT in all stratification groups, except for patients aged <50, patients with HER2+ disease and patients without comorbidities.

Conclusions

This study confirms superior BCSS and OS for BCT compared to mastectomy for most patients with DCIS or invasive breast cancer, irrespective of comorbidities and receptor status. This was confirmed both for long follow-up and following modern adjuvant therapy. A limitation is found in the observational design where confounding by indication or residual confounding cannot be excluded completely. However, based on the results clinicians seem right to prefer BCT in most patients with primary breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-12-01

Title: A phase II, open-label, multi-center study of ANG1005, a novel brain-penetrant peptide-drug conjugate, in breast cancer patients with recurrent CNS metastases

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Body: Background: The incidence of CNS metastatic disease in breast cancer patients (pts) seems to have increased in recent years with the improvement of systemic therapy. However, treatment options for CNS metastases have remained limited due to the inability of most agents to cross the BBB. ANG1005 is a novel peptide drug-conjugate, consisting of 3 paclitaxel molecules covalently linked to Angiopep-2, a peptide designed to utilize the LRP-1 transport system to cross the BBB/BCB and to penetrate malignant cells.

Methods: We conducted an open label phase II clinical study to test its activity in metastatic breast cancer (BC) pts with recurrent brain metastasis (BM), including BCBM pts with newly diagnosed leptomeningeal carcinomatosis (LC). Adult pts with measurable, recurrent BM from breast cancer, with or without LC (n=72 safety population; n=58 efficacy population) were enrolled in the study. ANG1005 was administered IV at 600 mg/m² q3w. HER2+ patients were allowed to continue trastuzumab +/- pertuzumab. Intracranial (IC) response was assessed by Gd-MRI using CNS RECIST 1.1 and extracranial response (EC) was evaluated per RECIST 1.1.

Results: Median age was 47.5 (26-76) years. Safety was similar to that of paclitaxel with myelosuppression as the predominant toxicity (WBC: 83%, RBC: 71%, PLT: 69%). Pts received a median of 6 (1-29) prior therapies for BC, including 84% with taxanes. As prior therapy for BM, 87% pts had cranial surgery and/or radiation and 19% pts received systemic therapies. Intracranial tumor response is presented for all pts as well as the various patient subsets as shown below:

Table 1: Intracranial Response by Breast Cancer Subset

Outcome by CNS RECIST	All	HER2+	HER2-	TNBC	LC
Sample size, n	58	28	30	12	23
PR (best response), n (%)	8 (14%)	4 (14%)	4 (13%)	2 (17%)	5 (22%)
Confirmed PR, n (%)	3 (5%)	2 (7%)	1 (3%)	0	2 (9%)
SD, n (%)	33 (57%)	19 (68%)	14 (47%)	5 (42%)	12 (52%)
PD, n (%)	17 (29%)	5 (18%)	12 (40%)	5 (42%)	6 (26%)
Clinical benefit (SD+PR), %	71%	82%	60%	59%	74%

Abbreviations: TNBC, triple-negative breast cancer, a sub-group of HER2-; LC, leptomeningeal carcinomatosis

Table 1 definitions: data based on evaluable pts with clinical or radiological evaluation \geq 4 weeks from C1D1; LC pts included 15 HER2+ and 8 HER2-; best response is the best response recorded from the start of the study treatment until the disease progression; confirmed PR requires a \geq 28-day confirmation of response.

Extracranial tumor responses of 1 (3%) CR, 2 (7%) PRs and 24 (80%) SDs were seen in 30 evaluable pts, including after prior taxanes (93%). The 6 month OS in all pts was 50.2% (95% CI: 37.4, 61.6).

In general, CNS clinical symptoms post-ANG1005 were improved, including in LC pts.

Conclusions: ANG1005 is active against previously treated BC metastasis both within and outside the CNS. A randomized study is planned. Updated efficacy and safety data will be presented at the meeting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-12-02

Title: Prognostic factors and survival according to tumor subtype in women presenting with breast cancer brain metastases at initial diagnosis

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Body: Background: Breast cancer represents one of the most common causes of brain metastases. The occurrence of this event is a catastrophic complication of metastatic breast cancer, associated with poor prognosis. However, the presence of brain metastases at the time of initial breast cancer diagnosis (BMIBCD) is uncommon. Because of this, the prognostic assessment and management of patients (pts) who present with BMIBCD is very challenging. The aim of this study was to analyze the influence of tumor subtype compared with other prognostic factors in the survival of pts who present with BMIBCD.

Methods: We evaluated women with brain metastases at the time of initial diagnosis of microscopically confirmed breast cancer, reported to the Surveillance, Epidemiology and End Results (SEER) 18 registries program from 2010 to 2013. Pts with other primary malignancy either before or after breast cancer were excluded. Pt characteristics were compared between tumor subtypes. Univariate and multivariate analyses were performed to determine the effects of each variable on overall survival (OS).

Results: Seven hundred forty pts were included. Median age was 60 years (range 26-93). After a median follow-up of 6 months (range 1-48), median OS for the whole population was 10 months and 20.7% of pts were alive at 36 months. At diagnosis, brain was the only site of metastasis in 125 pts, whereas 66.2% of pts had metastases in bone, 33.2% in liver and 44.7% in lung. Tumor subtype distribution was: 46.6% hormone receptor (HR)+/HER2-, 17% HR+/HER2+, 14.1% HR-/HER2+ and 22.3% triple negative (TN). Pts with TN brain metastases had higher grade ($p<0.0001$), higher rate of brain only ($p=0.001$) and lung metastases ($p=0.03$), had lower rates of bone metastases ($p<0.0001$) and were more likely to die from breast cancer ($p<0.0001$). Univariate analysis showed that older age ($p=0.0002$), black race ($p=0.004$), lobular histology ($p=0.01$), unmarried pts ($p=0.01$), the presence of liver ($p<0.0001$) and lung metastases ($p=0.002$) and TN pts (median OS 6 months; $p<0.0001$) had worse prognosis. The HR+/HER2+ subtype had the longest OS with a median of 22 months. In multivariate analysis, older age (hazard ratio 1.8; $p=0.001$), lobular histology (hazard ratio 2.08; $p=0.006$), TN subtype (hazard ratio 2.25; $p<0.001$), liver metastases (hazard ratio 1.6; $p=0.001$) and unmarried pts (hazard ratio 1.39; $p=0.04$) had significantly shorter OS. Race, tumor grade, bone metastases, lung metastases and brain only metastases had no association with OS.

Conclusions: To our knowledge, this is the largest study of BMIBCD. Although the prognosis of these pts is generally poor, it is important to notice that 20.7% of pts were still alive 3 years after diagnosis. There were substantial differences in OS according to tumor subtype, TN pts had the shortest OS. HR+/HER2- represented the largest proportion of cases, therefore these pts should not be ignored when designing clinical trials for pts with brain metastases. In addition to tumor subtype, other independent predictors of OS are age at diagnosis, marital status, histology and liver metastases.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-12-03

Title: Association between tumor genotype and development of brain metastases in patients with hormone receptor positive (HR+)/HER2- metastatic breast cancer

Fitzgerald DM M, Henderson LE E, Isakoff SJ J, Moy B, Oh K, Shih HA A, Dias-Santagata D, Borger DR R, Iafrate AJ J, Brastianos PK K, Bardia A and Juric D. Massachusetts General Hospital, Boston, MA.

Body: Introduction: Historically, brain metastases are considered to be uncommon in HR+/HER2- breast cancer compared to triple-negative or HER2+ breast cancer. However, improved systemic therapy and prolonged overall survival in patients with metastatic HR+/HER2- breast cancer may result in increased incidence of brain metastases as most currently available therapeutic agents do not penetrate blood-brain barrier giving the brain a sanctuary site status. Although certain tumor cells may also exhibit brain-specific tropism or may have selective growth advantage in the brain microenvironment, biological factors that govern metastases to brain, including role of *PIK3CA* mutations, are poorly understood. In this study, we review our clinical experience with the brain metastases among patients with metastatic ER+/HER2- breast cancer, including their association with *PIK3CA* genotype.

Methods: Since 2008, at our institution, a multiplexed tumor genotyping assay (SNaPshot), has been utilized to assess for presence of potentially actionable oncogenic driver mutations, including *PIK3CA*, using DNA derived from formalin-fixed, paraffin-embedded (FFPE) tissue. We identified patients with metastatic HR+/HER2- breast cancer who had tumor genotyping performed at any point during their care and who had at least 6 months of follow-up in our clinic. Relevant clinical information, including development of brain metastases, was gathered from chart reviews.

Results: From a total of 251 patients with HR+/HER2- metastatic breast cancer, 23.5% (N=59) developed brain metastases. Approximately 1/3rd of patients (31.7%, N = 20) had brain metastases seen on imaging as an incidental finding, while others presented with 1-2 symptoms that could be associated with CNS disease, including ataxia/weakness (34.9%), visual/speech difficulties (26.9%), headaches (23.8%), altered mental status (14.3%), seizures (14.3%), and nausea (9.5%). *PIK3CA* mutations were identified in 45.2% of all patients, including mutations in both helical (exon 9) and kinase (exon 20) domains. Patients with tumors harboring *PIK3CA* mutations had significantly higher incidence of brain metastases, as compared to those without *PIK3CA* mutations (30.7%, versus 18.7%; $p = 0.034$). The median time between diagnosis of metastatic disease and diagnosis of brain metastasis was longer among those patients with *PIK3CA* mutation (32 months) as compared to those without *PIK3CA* mutation (18 months).

Conclusion: Brain metastases are common among patients with HR+/HER2- breast cancer, particularly HR+/HER2- breast cancer harboring *PIK3CA* mutations where it approaches the incidence historically seen in HER2+ breast cancer. Early recognition and appropriate diagnostic work-up of any symptoms potentially associated with presence of CNS disease is necessary in *PIK3CA*-mutant HR+/HER2- breast cancer. Further studies are needed to explain the mechanistic link between the *PIK3CA* mutant phenotype, phosphatidylinositol 3-kinase (PI3K) pathway activation and CNS disease.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-12-04

Title: Phase I dose-escalation trial of ONT-380 in combination with trastuzumab in patients (pts) with HER2+ breast cancer brain metastases

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Body: Background: Brain metastases remain an important cause of death for pts with advanced HER2+ BC. ONT-380 is an oral, potent and selective inhibitor of HER2 capable of penetrating the blood brain barrier in pre-clinical models. We evaluated the safety and preliminary efficacy of ONT-380 in combination with trastuzumab.

Methods: Open-label, dose finding, Phase I study to estimate the MTD(s) of ONT-380 + trastuzumab in pts with HER2+ BCBM. The study was conducted in parallel cohorts testing two schedules of ONT-380 (once-daily or twice-daily). The primary endpoint was determination of the MTD. A total of 15 pts were to be treated at the MTD of each schedule in order to select the best regimen for future phase 2 studies. Response was assessed by RECIST 1.1 (extracranial) and modified RECIST 1.1 (intracranial).

Results: Between Aug 2013 and March 2016, 41 pts were enrolled. Median age was 49 (range 29-65); ECOG performance status 0-1 (85%), 2 (15%). 34 (83%) patients had progressed after prior WBRT and/or SRS. Patients had a median of 2 prior treatments for metastatic breast cancer prior to enrollment. As of June 7, 2016, median number of cycles was 4 (range 1-32); 4 patients remain on active protocol therapy. 2 DLTs were observed among 5 pts treated at 450 mg BID (G3 thrombocytopenia and G3 thrombocytopenia and G3 ALT). The dose was de-escalated to 300 mg BID, and with 0/3 patients experiencing a DLT. 300mg BID was identified as the MTD and an additional 14 patients were enrolled at this dose level. 1/7 pts experienced a DLT at 750 mg QD (G3 ALT). At 900 mg QD, 2/2 pts experienced a DLT (2 patients had G3 ALT/AST). An additional 10 pts were enrolled at the MTD of 750 mg QD. The most common AEs, regardless of relationship, were fatigue, diarrhea, AST/ALT elevation, nausea, headache. Gr 3/4 AST/ALT elevation has occurred in 9/41 pts (22%), though has been reversible with dose interruption and reduction. One Gr 3 diarrhea has occurred at 450 mg BID, and 1 Gr 3 diarrhea has occurred at 750 mg QD. PK assessments showed similar exposure of ONT-380 for QD and BID regimens. Preliminary assessment CNS response is available for 19 pts in the QD cohort (5% PR, 89% SD, 5% PD) and 17 pts in the BID cohort (12% PR, 59% SD, 12% PD, 18% unknown). Preliminary non-CNS assessment for the same population is as follows: QD cohort (100% SD); BID cohort (6% PR, 71% SD and 23% unknown).

Conclusions: The combination of ONT-380 and trastuzumab is feasible. The combination has an acceptable safety profile with low incidence and severity of diarrhea and rash. Transaminase elevation was asymptomatic for all pts and resolved with drug interruption/delay. Preliminary evidence of activity was observed in CNS and non-CNS disease.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-12-05

Title: Etirinotecan pegol: Survival advantage over standard of care drugs in a model of brain metastases of breast cancer

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Body: BACKGROUND: Brain metastasis of breast cancer is a significant cause of death among women with disseminated breast cancer. Chemotherapy, radiation, and surgery for these metastases provide only minimal benefit with considerable toxicity. The long-acting topoisomerase I inhibitor etirinotecan pegol (EP) is a novel treatment for disseminated breast cancer. EP consists of irinotecan attached to a 20kDa branched polymer via a releasable linker. The large molecular weight of EP results in tumor accumulation, including brain metastases, providing increased and sustained exposure to cytotoxic SN38. In the Phase 3 BEACON study, EP showed a significant survival benefit (HR 0.51; 95% CI 0.30-0.86) in a prespecified subgroup of women with history of treated, stable brain metastases compared to treatment of physicians choice (TPC) consisting of one of seven approved chemotherapy drugs (median OS 10.0 vs 4.8 mo). Nektar has filed a conditional marketing authorization in Europe for EP in patients with advanced breast cancer and brain metastases. A Phase 3 trial in this patient population is being initiated to support a potential U.S. filing. To further support the biological rationale for EP in these patients, an experimental preclinical model of brain metastases of breast cancer was conducted to compare the efficacy of EP to TPC in BEACON and the planned subsequent study.

METHODS: Female athymic nude mice were inoculated with 1.75×10^5 MDA-MB-231-BrLuc cells via intracardiac injection. Starting on day 21, gemcitabine (60 mg/kg) and eribulin (1.5 mg/kg) were dosed IP every 4 days; EP (50 mg/kg), irinotecan (50 mg/kg), paclitaxel (6 mg/kg), vinorelbine (10 mg/kg), docetaxel (10 mg/kg), and vehicle (saline or dextrose) were dosed weekly via tail vein. Efficacy was measured by tumor burden and survival. Tumor burden was determined based on twice weekly bioluminescence measurements. Animals were euthanized according to international animal care guidelines.

RESULTS: Median survival for all control arm groups (docetaxel, vinorelbine, eribulin, gemcitabine, irinotecan, and paclitaxel) were 39, 43, 41, 48, 35, and 42 days, respectively, none of which were significantly different from vehicle control median survival of 40 days ($p > 0.05$). No animals receiving these drugs survived until the end of the 90-day trial. Median survival for the EP treatment group was 86 days ($p < 0.05$) with a 40% survival rate at 90 days, the trial endpoint. Tumor burden increased nearly 100-fold in all TPC and vehicle groups, whereas EP significantly inhibited tumor growth. Quantitative autoradiography showed that EP accumulated ~10-fold in brain metastases, while accumulation in non-tumor brain tissue was only ~2-fold compared to irinotecan. EP at 50 mg/kg achieved plasma trough concentrations comparable to those observed in patients receiving the recommended dose of 145 mg/m² every 21 days.

CONCLUSIONS: In this model of brain metastases, EP preferentially accumulates in brain metastases, significantly reduces tumor burden progression and significantly improves survival in brain metastases of breast cancer compared to the most active chemotherapeutic agents available for advanced breast cancer and those used in the BEACON trial and planned subsequent Phase 3 study.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-12-06

Title: Factors related to the prognosis of breast cancer patients after the development of brain metastases

Griguolo G, Dieci MV, Giarratano T, Giorgi CA, Orvieto E, Ghiotto C, Falci C, Mioranza E, Tasca G, Milite N, Miglietta F, Conte P and Guarneri V. Medical Oncology 2, Istituto Oncologico Veneto IRCCS, Padova; University of Padova, Padova and Azienda Ospedaliera di Padova, Padova.

Body: Background: Brain metastases (BM) are a serious relatively common complication of breast cancer (BC). We evaluated prognostic factors for survival after diagnosis of BM from BC in a contemporary cohort of pts.

Methods: Pts diagnosed with BM from BC between 1999 and march 2016 and treated at the Istituto Oncologico Veneto of Padua were evaluated. Overall survival (OS) was defined as time from BM diagnosis to death or last follow-up. Pts were classified in 4 categories according to the breast cancer-specific Graded Prognostic Assessment (GPA) index according to validated criteria (Sperduto et al, 2012), based on age, Karnofsky Performance Status (KPS) and BC phenotype. Cox proportional models were used to calculate HR and 95% CI.

Results: 199 pts were identified. Median age at BM diagnosis was 56 yrs (range 28-84). Tumor phenotype distribution was as follows: triple negative (TN, 20.1%), hormone receptor (HR)-HER2+ (16.8%), HR+HER2+ (24.0%) and HR+HER2- (39.1%). Median time to BM diagnosis was 48.9 months (range 0-327), with significant differences according to tumor phenotype (median 27.3, 31.8, 46.1 and 55.2 months in TN, HR-HER2+, HR+HER2+, HR+HER2-, respectively, p=0.009). With respect to OS, no significant difference was observed across tumor phenotypes, with TN patients experiencing the worse outcome (median: 4.7, 7.7, 11.0 and 6.2 months in TN, HR-HER2+, HR+HER2+, HR+HER2-, p=0.187). The breast-specific GPA index, which combines tumor phenotype with patient-related features, was significantly associated with OS (Table). The number of local treatment received (radiotherapy, either whole brain or stereotactic, or neurosurgery) and the administration of systemic treatment after BM diagnosis were significantly associated with better OS (Table). Patients in the less favorable GPA category (GPA index ≤ 1) were less likely to receive systemic treatment after BM diagnosis compared to other GPA categories (43% vs 71%, p=0.009); no association between GPA category and local treatment was observed. Patients undergoing increased lines of local treatments were more likely to receive systemic therapy (chi2 square test p<0.001). To avoid bias we performed two separate multivariate analyses including: i) GPA category and number of local treatments; ii) GPA category (patients with GPA index ≤ 1 excluded) and systemic treatment. GPA maintained a significant prognostic value in both models (p=0.002 and p=0.038, respectively). Both local and systemic treatments added independent prognostication beyond GPA (Table).

Prognostic factors	Median OS, months (95%CI)	HR (95%CI), univariate	p, univariate	HR (95%CI), corrected for GPA
GPA category				
3.5-4	18.8 (15.2-22.5)	ref		-
2.5-3	8.8 (3.8-13.8)	1.40 (0.80-2.43)		-
1.5-2	5.5 (3.5-7.5)	1.76 (1.00-3.10)		-
0-1.0	2.7 (1.2-4.3)	2.67 (1.35-5.28)	0.019	-
Number of local treatments received				
0	3.0 (1.8-7.5)	ref		ref
1	8.0 (5.8-10.3)	0.53 (0.38-0.74)		0.54 (0.38-0.78)
2	21.3 (15.2-27.3)	0.36 (0.20-0.65)		0.49 (0.26-0.93)
3	35.5 (33.5-37.6)	0.12 (0.04-0.38)	<0.001	0.08 (0.02-0.33)
Systemic treatment				
yes	13.1 (8.7-17.4)			

no	2.6 (1.3-3.8)	0.42 (0.30-0.58)	<0.001	0.46 (0.31-0.68)
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Conclusions: Patient-related features, tumor phenotype and multimodal treatments all independently contribute to modulate the prognosis of pts with BM from BC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-12-07

Title: Phase Ib/II single-arm trial evaluating the combination of everolimus, lapatinib and capecitabine for the treatment of patients with HER2-positive metastatic breast cancer with progression in the CNS after trastuzumab (TRIO-US B-09)

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Body: Background: Improving outcomes for patients with HER2+ CNS metastases remains an unmet clinical need. Lapatinib (L) plus capecitabine (C) yields a 20% objective response rate (ORR) in the CNS in patients with previously treated HER2+ breast cancer brain metastases (Lin N, Clin Cancer Res 2009). Everolimus (E), an oral inhibitor of the mammalian target of rapamycin (mTOR), penetrates into the CNS in murine xenograft models (Meikle L, J Neurosci 2008). TRIO-US B09 is an investigator-initiated trial evaluating the safety and clinical activity of the novel combination of L+C+E for the treatment of patients with HER2+ breast cancer brain metastases.

Methods: Patients with trastuzumab-pretreated, HER2+ metastatic breast cancer (MBC) with progression of disease (PD) in the brain and a measurable brain lesion participated. Patients were excluded if they had a prior mTOR inhibitor or an ECOG PS>2. Prior L and/or C, and prior surgery and/or radiation to the brain were allowed. The primary endpoint was CNS ORR at 12 weeks (cycle 3) by RECIST 1.1. Secondary endpoints included safety, progression-free survival, overall survival and extra-CNS ORR. To test the safety of the combination of L+C+E, a 3+3 dose escalation phase was conducted (starting doses: L 1000 mg QD, E 5 mg QD, C: 750 mg/m² BID d1-14). Treatment was given Q21 days. Patients were evaluated for dose limiting toxicities during C1. Tumor imaging was conducted every 3 cycles. MRI of the brain was performed every 2 cycles through cycle 6 and then every 3 cycles. Neurological symptom assessment was conducted on day 1 of every cycle. Study participants continued to receive treatment until PD, unacceptable toxicity or withdrawal of consent for 12 mos.

Results: Nineteen patients were enrolled at 11 sites in the US and treated with at least one dose of study drug. Of 18 patients with data available, median age was 58.5 (45-68), median number of systemic therapies for MBC was 2 (0-6), and 94.4% had prior radiation and/or surgical resection of brain metastases. 10 patients participated in the dose escalation phase of the study. The maximum tolerated doses were determined to be L 1000 mg QD, E 10 mg QD + C 1000 mg/m²BID days 1-14; however, given tolerability concerns, dose expansion proceeded with Cohort 2 dose for C (750 mg/m² BID d1-14). Of 17 eligible patients with imaging results available to date, 2 (12%) had a partial response in the CNS at week 12, one of whom continues on study (currently in cycle 13). Stable disease was observed in 7 patients. The most common grade 3/4 adverse events (AE) (CTCAE v4.0) related to E and/or L in 18 treated patients were anorexia (5.5%), dehydration (5.5%), diarrhea (17%), fatigue (5.5%), fever (5.5%) hyperglycemia (5.5%), hypokalemia (11%), and oral mucositis (17%).

Conclusions: This is the first report of this regimen for patients with HER2+ MBC to the brain. This regimen is generally well-tolerated and shows promising activity in the CNS of heavily pretreated patients. Final efficacy and toxicity analyses for all 19 patients will be presented.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-12-08

Title: The incidence and outcomes of brain metastases in HER2-positive metastatic breast cancer with the advent of modern anti-HER2 therapies

Strulov Shachar S, Deal AM M, Vaz-Luis I, Dees EC Claire, Carey LA A, Hassett MJ J, Garrett AL L, Benbow JM M, Hughes ME E, Mounsey L, Lin N and Anders CK K. Lineberger Comprehensive Cancer Center, Chapel Hill, NC; Rambam Health Care Campus, Haifa, Israel and Dana-Farber Cancer Institute, Boston, MA.

Body: Background: Human epidermal growth factor receptor 2 (HER2) is over-expressed in approximately 20 - 30% of breast cancers. HER2-positive breast cancers frequently metastasize to the brain. In recent years, many new drugs have been approved for HER2-positive metastatic breast cancer (MBC). In the metastatic setting, trastuzumab was approved in 2000, lapatinib 2007, and pertuzumab and ado-trastuzumab emtansine in 2012. We sought to describe the incidence, time course, and prognostic factors of BM in patients (pts) with HER2+ MBC during the time when dramatic changes in systemic therapy occurred.

Patients/methods: The study included pts with HER2-positive MBC treated at two academic hospitals: Dana Farber Cancer Institute (DFCI) (2000-2007 [DFCI-T1], 2008-2011 [DFCI-T2]) and University of North Carolina (UNC) (2012-2014). We examined the incidence of BM (at diagnosis [dx] and within 1-2 years of MBC dx). We combined the two cohorts to examine outcomes – time to BM, survival following MBC, and survival following BM – using the Kaplan Meier method and Cox regression modeling.

Results: We identified 185 (DFCI n=128, 97 diagnosed 2000-2007 and 31 diagnosed 2008-2011; UNC n=57, all diagnosed 2012-2014) pts with HER2-positive MBC. Through a median of 4 years follow-up after the MBC dx (min 2, max 11), 118 had died and 67 were censored. The median age at MBC dx was 52 (min 25, max 88), 149 (82%) were Caucasian, 88 (48%) had hormone receptor (HR) positive BC, and 67 (37%) had de-novo (i.e., non-recurrent) MBC. BM was present at the MBC dx for 8% of pts in DFCI-T1, 16 % of pts in DFCI-T2, and 16% of pts at UNC. Within 1 year of the MBC dx, BM was present in 21% of DFCI-T1, 29% in DFCI-T2, 23% of UNC pts. Within 2 years of the MBC dx, 67 (36%) pts had developed BM, of which one third (22) were diagnosed at initial MBC presentation. In unadjusted analyses, there were no differences in time to BM dx by age ($p=0.2$), race ($p=0.1$) or HR status ($p=0.1$). The median survival following the development of BM for all pts was 1.5 years. A multivariable model predicting survival after the MBC dx, found factors associated with shorter survival included having (vs. not having) BM at the initial MBC dx, having received (vs. not having received) adjuvant HER2-directed therapy prior to the MBC dx, and having recurrent (vs. de novo) MBC ($P\leq 0.02$ for all). Age, HR status, race and time period of MBC dx were not significant in the multivariable model.

Conclusions: Among pts diagnosed in the modern era, after new therapies became available, BM remains a common problem for pts with HER2-positive MBC. While no obvious trends in the incidence of HER2-positive MBC are suggested, conclusions regarding incidence trends should be considered hypothesis-generating until larger, population-based data become available. Nevertheless, a dx of BM early in the course of MBC treatment and prior receipt of adjuvant trastuzumab appeared to confer a more aggressive disease course. Coordinated, prospective collection of the incidence and outcomes of BM among pts with HER2-positive MBC, studies of pts who develop BM >2 years after their MBC dx, and clinical trials of treatment strategies for pts with trastuzumab-resistant BM are needed.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-12-09

Title: Clinical characteristics of CNS metastases of different breast cancer subtypes – Results from a cohort study

Lindman H, Nilsson A and Gullbo J. Uppsala University Hospital, Uppsala, Sweden.

Body: Introduction: Secondary breast cancer in CNS is a rising clinical problem despite an ongoing improvement of the prognosis in breast cancer in general. The commonly proposed reason is the decreased passage through the blood-brain-barrier (BBB) of otherwise effective systemic therapies. The prognosis of patients (pts) with metastases (met) in CNS is poor and the therapy is mainly based on local therapy with surgery or stereotactic radiotherapy or alternatively, whole brain radiotherapy. However, therapy differs among centers and guidelines are based on relatively small studies with inhomogeneous groups of pts. The value of systemic therapy is not clearly established. Data has shown different outcome in different breast cancer subtypes.

Patients and Methods: Pts treated between 2009 and 2016 with metastases in CNS (brain, medulla and/or meningeal) were identified in our treatment database. This represents all breast cancer pts in Uppsala County with CNS involvement during that time period. We categorized the pts based on number of brain met and on the tumor subtype. We compared the pattern of metastases, the relationship to extra-CNS met and time, response to given therapy and survival.

Results: Of 68 pts 51% had oligometastases in brain, 41% multiple brain metastases and 7% meningeal metastases only. Median age was 58 years (range 34-81) median size of largest tumor was 16 mm. The most common subtype was HER2 positive (HER2) 37% followed by Luminal B (LumB) 31%, Triple negative (TNBC) 18% and Luminal A (LumA) 15%. The subtypes had different clinical appearance according to table:

	HER2 (n=25)	TNBC (n=12)	LumB (n=21)	LumA (n=10)
Multiple met (>3)	44%	9%	48%	60%
Oligo met (1-3)	56%	91%	38%	30%
Meningeal only	0%	9%	14%	10%
Extra CNS met at diagnosis	76%	67%	81%	100%
Median time from M1 to CNS met (months)	3	0	22	45
Median survival (months)	24	5.5	14	6

In more than 50% of the TNBC pts was CNS the first site of disseminated disease. This was the case in only 24% of the LumB pts and none of the LumA pts. Systemic therapies were given to 91% of all pts and radiological complete responses in CNS were reported in 24% (HER2 32%, TNBC 17%, LumB 24% and LumA 10%). The median survival was 13 months, but differed significantly between the different subtypes and was surprisingly short for LumA, which not could be explained by death by metastases outside CNS. Eventually, developed 25% of all pts meningeal involvement. CNS met were the prime reason for death in 72% of the pts.

Conclusions: This cohort study describes the clinical appearance and prognosis of CNS metastases in breast cancer pts during a seven years period in the institution treating all pats in the County of Uppsala. Distinctive clinical differences between the breast cancer subtypes could be noticed which possible represents different mechanism in the metastatic process as well as different treatment response. The survival in the luminal subgroups was short and CNS met developed considerably later in the course of the disease when pts might have developed a more aggressive and therapy resistant disease.

2016 San Antonio Breast Cancer Symposium

Publication Number: P1-12-10

Title: Safety and efficacy of trastuzumab emtansine (T-DM1) in 399 patients with central nervous system metastases: Exploratory subgroup analysis from the KAMILLA study

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Body: Introduction T-DM1 treatment significantly improved overall survival and had a lower incidence of grade ≥ 3 adverse events (AEs) vs capecitabine plus lapatinib in patients (pts) with HER2-positive advanced breast cancer (BC) in the EMILIA study, including pts with treated, asymptomatic CNS metastases (mets). KAMILLA is an ongoing, single-arm, open-label, phase 3b global safety study of T-DM1 in pts with HER2-positive locally-advanced or metastatic BC (target n=2220). In this interim analysis we describe clinical characteristics, safety, and efficacy in pts with stable CNS mets at baseline (BL).

Methods Eligible pts received prior HER2-directed therapy and chemotherapy and progressed on or after most recent treatment for advanced BC, or within 6 months of completing adjuvant therapy. Pts with asymptomatic CNS mets were eligible, including pts with stable CNS disease with prior radiation therapy. Pts received T-DM1 3.6 mg/kg every 3 weeks until unacceptable toxicity, withdrawal of consent, or disease progression. This exploratory analysis describes pts with BL CNS mets. Median progression-free survival (PFS) was estimated using the Kaplan-Meier method.

Results As of April 4, 2016, data were available for 2017 treated pts, of whom 399 (20%) had BL CNS mets, with a median follow-up of 33 months. Table 1 presents demographic and BL characteristics.

The incidence of AEs was similar between pts with and without BL CNS mets. Serious AEs (SAEs) occurred in 112 pts with BL CNS mets (28%) vs 311 of 1618 pts without BL CNS mets (19%), and the frequency of SAEs was comparable to that observed in EMILIA. However, nervous system disorder SAEs, such as seizure, epilepsy, and brain edema, occurred more frequently in pts with BL CNS mets (30 pts [8%]) vs 18 pts [1%] with no BL CNS mets.

Mean T-DM1 treatment duration was 8.0 months (median 8 cycles; range 1–55) in pts with BL CNS mets and 9.4 months (median 9 cycles; range 1–57) in pts with no BL CNS mets. Median PFS was 5.5 months in pts with BL CNS mets vs 7.9 months in pts with no BL CNS mets. A decrease in the size of brain target lesions was observed during T-DM1 treatment in 84 of 126 pts with measurable CNS lesions.

Table 1. BL characteristics

	CNS mets at BL (n=399)	No BL CNS mets (n=1618)
Median age, yrs (range)	52 (28–83)	55 (26–88)
ECOG performance status, n (%)		
0	193 (48)	929 (57)
1	174 (44)	605 (37)
2	32 (8)	83 (5)
Hormone receptor status, n (%)		
ER and/or PR positive	246 (62)	992 (61)
ER and PR negative	149 (37)	593 (37)
Median time since initial BC diagnosis, yrs (range)	4.8 (0–28)	5.0 (0–53)
Median time since first metastasis, yrs (range)	2.4 (0–25)	2.6 (0–35)
Median number of prior therapies for metastatic BC (range)	3 (0–>10)	2 (0–>10)

Conclusions This subgroup analysis of KAMILLA is the largest reported cohort of pts with CNS mets treated with T-DM1. The overall safety profile of T-DM1 in pts with BL CNS mets was comparable to that of pts without CNS mets. As might be expected in pts with CNS disease, serious neurological AEs occurred more frequently in pts with BL CNS mets vs those without. Response to T-DM1 was seen in the CNS in pts with BL CNS mets, however, median PFS was lower in pts with BL CNS mets vs those without BL CNS mets.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD1-01

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: PD1-02

Title: Circulating tumor DNA (ctDNA): A *real-time* application of precision medicine to the management of metastatic breast cancer (MBC)

Rossi G, Austin LK K, Nagy RJ J, Rademaker AW W, Gradishar WJ J, Santa-Maria CA A, Curry-Edwards RL L, Jain S, Flaum LE E, Lima Barros Costa R, Zagonel V, Platanius LC C, Giles FJ J, Talasz A and Cristofanilli M. U.O.C. Oncologia Medica 1 - Istituto Oncologico Veneto- IOV IRCCS, Padova, PD, Italy; Thomas Jefferson University Hospital, Philadelphia, PA; Guardant Health, Inc, Redwood City, CA; Northwestern University, Feinberg School of Medicine, Chicago, IL; Robert H Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL and Northwestern Medicine, Chicago, IL.

Body: Background: Molecular diagnostic, in particular next-generation sequencing (NGS) technologies, improved the detection of actionable mutations (mut) in MBC at baseline and recurrence. We evaluated the ability of ctDNA to detect molecular abnormalities, monitor disease progression and predict outcome.

Methods: We conducted a retrospective study of 91 patients (pts) with locally advanced and MBC, who had longitudinal assessment of their disease by ctDNA analysis. The plasma-based assay was performed utilizing Guardant360 (Guardant Health, CA), a digital NGS technology to sequence a panel of > 50 cancer genes. After tabulating number of muts and quantification of overall ctDNA detected for every patient at baseline, a receiver operating characteristic (ROC) analysis was performed to identify the best cut-offs that separated the pts who had a disease progression from those who hadn't, and the patients who died from those still alive. The overall survival (OS) analysis has been performed using Kaplan-Meier curves.

Results: 84 pts (92%) had stage IV cancer. 63% cases were ER+, 27% HER2+, 29% TNBC. 277 blood samples were collected and 84% had muts. 65% of the pts had serial samples. The average number of alterations detected in each sample was 3 (0-27) and the average ctDNA fraction detected was 4.5% (0-88.2%). The most common alterations were: *TP53* (52%), *PIK3CA* (40%), *ERBB2* (20%), *NOTCH1* (15.5%), *APC* (14%), *MET* (13%). 16 pts (19%) were initiated on a targeted therapy based on ctDNA test results. At the time of analysis 36 pts (39.6%) were dead, 55 (60.4%) were currently alive. PFS was 5.2 months (ms) and OS was 21.5 ms. A statistically significant difference in PFS and OS by log rank test was found between % ctDNA at baseline < 0.5 versus ≥ 0.5 ($p = 0.003$ and $p = 0.012$, respectively) and number of muts at baseline < 2 versus ≥ 2 ($p = 0.059$ borderline and $p = 0.0015$). Moreover, a statistically significant association by Fisher's exact test was found between the number of alterations and the % ctDNA detected in the baseline sample (% of pts with muts ≥ 2 was 19% when % ctDNA < 0.5%, versus 85% when % ctDNA ≥ 0.5 %; $p < 0.0001$).

PFS (ms) $p = 0.059$ (log rank test)	Muts < 2 (n = 32)	Muts ≥ 2 (n = 58)
6	58%	40%
12	30%	13%
18	21%	6%
24	-	-
PFS (ms) $p = 0.003$ (log rank test)	% ctDNA < 0.5 (n = 27)	% ctDNA ≥ 0.5 (n = 60)
6	65%	39%
12	41%	10%
18	23%	6%
24	-	-

OS (ms) p = 0.002 (log rank test)	Muts < 2 (n = 32)	Muts ≥ 2 (n = 57)
6	97%	66%
12	88%	51%
18	88%	42%
24	-	29%
OS (ms) p = 0.012 (log rank test)	% ctDNA < 0.5 (n = 27)	% ctDNA ≥ 0.5 (n = 59)
6	96%	69%
12	90%	55%
18	85%	48%
24	-	35%

Conclusions: ctDNA liquid biopsy provides a *real-time*, quantitative NGS-based assessment of MBC which is useful for treatment planning, disease monitoring and prognostic evaluation. Future prospective studies should consider the use of ctDNA for molecular and prognostic stratification.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD1-03

Title: Multivariate analysis of subtype and gene expression signatures predictive of pathologic complete response (pCR) in triple-negative breast cancer (TNBC): CALGB 40603 (Alliance)

Hoadley KA A, Hyslop T, Fan C, Berry DA A, Hahn O, Tolaney SM M, Sikov WM M, Perou CM M and Carey LA A. The University of North Carolina at Chapel Hill, Chapel Hill, NC; Alliance Statistics and Data Center, Duke University, Durham, NC; Alliance Statistics and Data Center, MD Anderson Cancer Center, Houston, TX; Alliance Protocol Operations Office, University of Chicago, Chicago, IL; Dana-Farber/Partners CancerCare, Boston, MA and Women and Infants Hospital of Rhode Island, Providence, RI.

Body: Support: U10CA180821, U10CA180882

Background: The addition of either carboplatin (Cb) or bevacizumab (Bev) to standard neoadjuvant chemotherapy (NACT) increases pCR rates in TNBC overall and in the dominant subset of basal-like cancers (Sikov et al, JCO 2015; Sikov et al, SABCS 2014). Multigene expression signatures more accurately reflect tumor biology for response prediction and prognosis than individual gene expression. We evaluated the ability of multivariate analysis of gene expression signatures to create predictive models for achievement of pCR in TNBC.

Methods: RNA sequencing was successful on 389 pretreatment samples from patients with available pCR data, and used to assign PAM50 subtype and calculate gene signatures scores for 489 published expression signatures. Elastic net, a penalized regression model for high dimensional variable selection, was used to select features associated with pCR in all TNBC and in the basal-like subset. Models were derived in a training set (2/3 of samples) and validated in a separate test set (1/3). A separate model was derived using 196 TNBC samples from patients treated only on the standard NACT +/- Cb arms for application to external TNBC neoadjuvant data sets not treated with Bev.

Results: Consistent with our prior partial data set, 343 (88%) of the cancers were classified basal-like, in whom the in breast pCR rate was 54%; the remainder were classified normal-like (n=32) or HER2-enriched (n=14) with a non-basal pCR rate of 56%. Elastic Net analysis in all TNBC generated a model of 23 signatures and treatment assignment with 68% sensitivity and 64% specificity. The area under the curve was 0.64 (p-value=0.0019). Nineteen modules, including immune cell signatures (Th1, NK, IgG), immunoglobulin variable region expression, addition of Cb and Bev and expression of genes at regions 15q25, 17p11.2-13.3, and 8p22 were positively associated with response. The latter two regions are associated with aggressive breast cancer, and while not part of the 17p13 signature, this region contains TP53, a gene important in TNBC. Six modules were associated with resistance, including luminal progenitor, TGFB, NOTCH, FOS/JUN, 8p amplicon, and eosinophil signatures. When limited to basal-like samples, a model including 32 modules and addition of Cb and Bev was generated, with 62.3% sensitivity and 59.1% specificity. Seventeen features were selected in both models. Omitting Bev-treated patients, a model using just the gene expression signatures was developed. The predictive value of this model will be assessed using an external cohort of TNBC patients treated with neoadjuvant docetaxel and Cb (NCT01560663) and results presented.

Conclusions: Multivariate analysis of gene expression signatures derived from pretreatment samples enabled the construction of models to predict achievement of pCR in TNBC. These models performed well on our test set, and will be assessed for their predictive ability in other TNBC data sets. If validated by future analyses, this could help us identify patients likely to achieve pCR with standard NACT and may benefit from the addition of agents such as Cb or Bev.

ClinicalTrials.gov Identifier: NCT00861705.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD1-04

Title: CSMD1 SNPs selectively affect anastrozole response in postmenopausal breast cancer patients

Cairns J, Ingle J, Dudenkov T, Kalari K, Buzdar A, Kubo M, Robson M, Ellis M, Goss P, Shepherd L, Goetz M, Weinshilboum R and Wang L. Mayo Clinic, Rochester, MN; The University of Texas MD Anderson Cancer Center, Houston, TX; Riken Center for Integrative Medical Science, Yokohama, Japan; Memorial Sloan Kettering Cancer Center, New York, NY; Baylor Cancer Center, Houston, TX; Massachusetts General Hospital, Boston, MA and NCIC Clinical Trials Group, Kingston, ON, Canada.

Body: **BACKGROUND:** Based on prospective clinical trials, there is no evidence for differences in efficacy between the 3 aromatase inhibitors (AIs) anastrozole, exemestane, and letrozole. The purpose of this study was to identify germline genetic variants associated with response to AIs and to help identify novel mechanisms associated with drug disease efficacy.

METHODS: A genome-wide association study (GWAS) was performed for 624 patients (Steroids 2015;99:32-38) to identify SNPs associated with estrogen level change in women with estrogen receptor (ER) positive breast cancer treated with anastrozole.

Replication of associated SNPs was performed in a GWAS from the MA.27 trial that compared adjuvant anastrozole and exemestane treatment of post-menopausal women with ER+ breast cancer. Functional studies were subsequently performed to determine SNP effects and underlying mechanisms.

RESULTS: Our initial GWAS identified SNPs within *CSMD1* that were associated with changes in estrogen levels during anastrozole therapy. An additional SNP in *CSMD1* was also associated with breast cancer events in CCTG MA.27. Functionally, we showed that *CSMD1* regulates *CYP19* expression in a SNP-, and in an anastrozole- dependent fashion. These phenomena were not observed for either letrozole or exemestane. In MA.27, an anastrozole- specific effect was also seen with the minor allele having a protective effect on time to distant metastasis (HR=0.49, p=0.00259), but this was not the case for exemestane (HR=0.71, p=0.111). Our *in vitro* functional studies indicated that overexpression of *CSMD1* sensitized anastrozole or letrozole resistant cells to anastrozole but not to the other two AIs. The SNP in *CSMD1* that was associated with increased *CSMD1* and *CYP19* expression levels increased anastrozole sensitivity, but not letrozole or exemestane in lymphoblastoid cell lines (LCLs) homozygous for either WT or variant *CSMD1* SNP genotypes. Based on these observations, we explored whether anastrozole has additional mechanisms beyond its function as a *CYP19* inhibitor. Utilizing an estrogen response element (ERE) luciferase reporter assay in a *CYP19* CRISPR knockout breast cancer T47D cell line and a surface plasmon resonance (SPR) assay, we found that anastrozole can also function as an ER α agonist, and can bind to, and result in, proteasome dependent ER α degradation, especially in the presence of E2. Treatment of these *CYP19* CRISPR knockout cells with anastrozole in the presence of increasing concentrations of E2 results in greater sensitivity compared with anastrozole alone, while the addition of E2, as expected, does not improve letrozole or exemestane sensitivity. These same observations were also seen in letrozole and anastrozole resistant cells.

CONCLUSIONS: Our findings suggest that anastrozole might be more effective than letrozole or exemestane in patients with the *CSMD1* SNP. Furthermore, anastrozole can function as an ER α agonist, binding to ER α and resulting in its degradation, especially in the presence of E2. These findings should help to make it possible to develop precision endocrine therapies for women who are candidates for AIs.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD1-05

Title: Breast cancer brain metastases show limited intrinsic subtype switching, yet exhibit acquired *ERBB2* amplifications and activating mutations

Priedigkeit N, Hartmaier RJ J, Chen Y, Vareslija D, Basudan A, Thomas R, Leone JP P, Lucas PC C, Bhargava R, Hamilton RL L, Chmielecki J, Davidson NE E, Oesterreich S, Brufsky AM M, Young L and Lee AV V. University of Pittsburgh, Pittsburgh, PA; Foundation Medicine Inc., Cambridge, MA; Royal College of Surgeons, Dublin, Leinster, Ireland and University of Iowa, Iowa City, IA.

Body: BACKGROUND: Metastasis is the major cause of mortality in breast cancer (BrCa) patients. Our understanding of brain metastasis (BrM) is limited, reflected by a lack of effective treatments. We aimed to (1) determine BrCa gene signature differences between primary tumors and matched BrM and (2) uncover BrM-specific alterations that may be clinically actionable.

MATERIALS and METHODS: NanoString expression profiling of 127 genes from 5 major prognostic tests (MammaPrint, EndoPredict, PAM50, OncotypeDX, MGI) was performed on 20 patient-matched primary (10 ER-neg, 10 ER-pos) and metastatic brain tumors. Subtype classification was performed using *genefu*. Protein changes in ER and HER2 (*ERBB2*) were confirmed by IHC. BrM-specific *ERBB2* gains were corroborated in a publicly available dataset of 18 additional patient-matched cases (dbGAP phs000730.v1.p1). To test whether *ERBB2* amplification and base pair mutation is metastasis-site specific, we further analyzed an expanded cohort of 7,884 breast tumors enriched for metastatic samples (52%) including liver (16.7%), lung (4.3%), bone (3.6%), and brain (2.0%) using comprehensive hybrid-capture sequencing of *ERBB2*.

RESULTS: 17/20 BrM retained the PAM50 subtype of the primary BrCa. Despite this concordance, 17/20 BrM harbored expression changes (< or > 2-fold) in clinically actionable genes including gains of *FGFR4* (30%), *FLT1* (20%), *AURKA* (10%) and loss of *ESR1* expression (45%). The most recurrently upregulated gene was *ERBB2*, showing a >2-fold expression increase in 35% of BrM. 3 of 13 (23.3%) cases originally HER2-negative, and thus HER2-therapy naive, in the primary BrCa were IHC-positive (3+) in the paired BrM with an observed metastasis-specific amplification of the *ERBB2* locus. In an independent dataset, 2 of 9 (22.2%) HER2-negative BrCa switched to HER2-positive with one BrM acquiring *ERBB2* amplification and the other showing metastatic enrichment of the activating V777L *ERBB2* mutation. Analysis of a large cohort of breast tumors (n=7,884) showed that across all organs *ERBB2* amplification and/or base pair mutation was similar (p=0.18) between primary (13%) and metastatic disease (12%), however, a strong and significant enrichment was seen for BrM (primary 13% vs BrM 24%, p<0.0005).

CONCLUSIONS: Taken together, these results demonstrate that the majority (85%) of patient-matched BrM retain the intrinsic subtype of the primary cancer. However, despite this transcriptional similarity, alterations in clinically actionable genes are common, with BrM acquiring *ERBB2* amplifications and/or base pair mutations at a frequency of ~20%, even in HER2-therapy naive tumors. In a large cohort of primary and metastatic breast cancers, there is also a unique enrichment for *ERBB2* alterations in BrM. This study provides a strong rationale to molecularly profile metastatic lesions to both better understand biological mechanisms of metastases and to perhaps refine therapeutic decision-making in advanced cancers.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD1-06

Title: Comparison of mutational landscapes of primary breast cancer and first metastatic relapse: Results from the ESOPE study

Cottu PH H, Boulai A, Callens C, Baulande S, Legoux-Ne P, Bernard V, Vincent-Salomon A, Benhamo V, Brain EGC GC, Chemlali W, Campone M, Bachelot TD D, Giacchetti S, Bonnetterre J, Bidard F-C, Servois V, Comte A, Belin L, Sigal B and Bièche I. Institut Curie, Paris, France; Institut Curie, Saint Cloud, France; Institut de Cancerologie de l'Ouest, Saint Herblain, France; Centre Leon Berard, Lyon, France; CHU Saint Louis, Paris, France and Centre Oscar Lambret, Lille, France.

Body: Background

Genomic profile of breast cancer metastases (M) may differ from that of the primary tumor (PT). In a multicenter prospective study (ESOPE, NCT 01956552) including 130 patients with biopsies of the first metastatic deposit, we have shown that luminal breast cancers are the most prone to phenotypical subtype changes (Comte et al, ASCO 2016#550). We report here the first results of a comparative PT/M targeted next generation sequencing (NGS) mutational analysis.

Methods

Of 130 patients, 117 paired PT/M samples obtained before any treatment were available for analysis. Targeted Sequencing was done using Illumina HiSeq2500 technology with a custom made 95 breast cancer associated genes panel. Sequence data were aligned to the human reference genome (hg19) using Bowtie2 algorithm. Median depth was 607X and 87% of targets achieved 100X depth. SNVs and indels were called using GATK UnifiedGenotyper. We retained COSMIC confirmed non synonymous, exonic/splice variants and observed at a frequency lower than 0,1% in population. Further confirmation of detected variants was performed with comparison to public databases (cbioportal, tumorportal), and potential pathogenicity was evaluated with 4 different public algorithms. We present here the results obtained from the first 35 matched PT/M samples (liver mets 68%), focusing analysis on 40 genes including PIK3CA (20 genes), ER (6 genes) and MAPK (11 genes) pathways, *RUNX1*, *CDH1* and *TP53* genes.

Results

Patients characteristics are representative of patients with first line metastatic breast cancer (Comte et al, ASCO 2016#550). Among the 40 genes analyzed in the 70 samples, we detected 134 somatic mutations (70 in PT and 64 in M) including 15 indels and 119 SNV. Among these 134 mutations there were 74 different mutations (66SNV and 8 indels) classified pathogenic for 26 and of unknown pathogenicity for 48 of them. We detected at least 1 mutation in 31 PT and in 28 M. Median numbers of mutations were 1 in PT (range 1-9) and 1 in M (range1-22) samples ($p=0.295$, Wilcoxon rank sum test). Top ten mutated genes in PT included *PIK3CA*, *TP53*, *NCOR1*, *NF1*, *GATA3*, *CDH1*, *ERBB3*, *PTEN*, *HRAS*, *INPP4B*. In M samples, the 10 top genes were *PIK3CA*, *TP53*, *ERBB3*, *AKT3*, *CDH1*, *ERBB4*, *GATA3*, *INPP4B*, *MET*, *MTOR*. Only 3 *ESR1* mutations were detected, including 1 PT/M pair and 1 M. Beyond highly shared *PIK3CA* and *TP53* mutations, overall crude PT/M discordance rate was 31%. Analysis by histological subtypes showed PT and M specific mutational profiles, suggesting a role in ERB gene family (notably *ERBB3*) and MAPK driven pathways in early metastatic progression. Specific metastatic site analysis suggested enrichment in MAPK pathway mutations in liver metastases when compared to other sites. Variant allelic fractions were globally not significantly different between PT and M samples.

Conclusion

In this prospective multicenter series of systematic biopsies of first metastases, we report a targeted mutational analysis of matched PT and M samples not modified by previous therapy exposure. Early analyses suggest specific genotypical changes according to tumor subtype and/or metastatic site. Extended and updated results will be reported at the meeting.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD1-07

Title: Comprehensive characterization of matched pre-treatment biopsies and residual disease of doxorubicin treated breast cancer

Lips EH H, Hoogstraat M, Mulder L, Nederlof PM M, Sonke GS S, Rodenhuis S, Wesseling J and Wessels LFA FA. The Netherlands Cancer Institute- Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands.

Body: Background

Neoadjuvant chemotherapy is standard of care for locally advanced breast cancer. Unfortunately not all patients benefit from this treatment. Even after decades of research, we still cannot predict which tumor will or will not respond. This may in part be due to tumor heterogeneity, as the sample taken before treatment not necessarily represents the tumor cell population that causes therapy resistance.

Methods

To test this hypothesis, we collected pre-treatment biopsies, resection specimens, and matched blood from 21 breast cancer patients treated with doxorubicin and cyclophosphamide in a neoadjuvant setting. Specifically, tumors were selected with a tumor percentage >50% after chemotherapy to enrich for resistant samples and ensure high quality data. RNA and whole exome sequencing were performed to characterize somatic mutations, copy number alterations and gene expression profiles. Histopathological characteristics were determined to obtain a comprehensive profile of all tumor samples.

Results

The comparisons of somatic variants and copy number alterations revealed a very diverse image: in several cases, high-level amplifications, large genomic gains or losses, and mutations in known oncogenes or tumor suppressors such as MAP3K1 and RUNX1 were either lost or gained during treatment, while in other cases no such changes were detected. We observed a remarkable number of genetic alterations involved in cell cycle progression and DNA damage checkpoints, including amplification of MDM2, CCND1 and CDK4, and copy number loss or mutations in CDKN1B and ATM. Strikingly, both cases of CDKN1B loss were identified in pre-treatment biopsies and no longer detectable in the surgery specimen. In contrast, CCND1, CDK4 and MDM2 amplifications were retained, although CCND1 expression decreased significantly in CCND1 amplified tumors. In addition, eighty percent of tumors showed a decreased cell proliferation after chemotherapy, where the high-proliferative ER+ (Luminal B) tumors were most strongly affected. This trend was also visible in a validation cohort of 94 ER+ samples, but the prognosis of Luminal B tumors that showed a decrease in proliferation was still significantly worse than that of Luminal A tumors that did not show an altered proliferation rate.

Conclusion

Our results confirm that biologically relevant genomic alterations can differ between pre- and post-treatment samples, which greatly impacts biomarker discovery. In addition, our findings emphasize the chemotherapy insensitivity of CCND1 amplified ER+ breast cancers, and stress the need for better treatment regimens for these patients. In contrast, genomic loss of CDKN1B may be a marker for sensitivity to doxorubicin.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD1-08

Title: High-throughput genome analysis and therapeutic decision for patients with HER2-negative metastatic breast cancer: First feasibility and molecular results of the randomized phase II study SAFIR02 BREAST (UCBG-0105/1304)

Gonçalves A, Bachelot T, Lusque A, Arnedos M, Campone M, Bièche I, Lacroix L, Pierron G, Dalenc F, Filleron T, Sablin M-P, Jimenez M, Ferrero J-M, Lefeuvre-Plesse C, Bonnefoi H, Attignon V, Soubeyran I, Jezequel P, Commo F and André F. Institut Paoli Calmettes, Marseille, France; Centre Léon Bérard, Lyon, France; Institut Claudius Regaud- IUCT-O, Toulouse, France; Gustave Roussy Cancer Campus, Villejuif, France; Institut de Cancérologie de l'Ouest, Nantes, France; Institut Curie, Paris, France; Unicancer, Paris, France; Centre Antoine Lacassagne, Nice, France; Centre Eugène Marquis, Rennes, France and Institut Bergonié, Bordeaux, France.

Body: Background A genomic-driven therapeutic strategy in metastatic breast cancer (MBC) was recently demonstrated as feasible in the clinical practice, but its actual impact on patient outcome remains elusive. SAFIR02 study is an ongoing national multicentric phase II randomized trial evaluating targeted therapies matching specific genomic alterations (GA) administered as maintenance after objective response and/or stable disease obtained with chemotherapy in HER2-negative MBC patients. This analysis reports on feasibility of the procedure and the rate of identified actionable targets.

Methods Eligible MBC patients (PS=0/1, first- or second-line of chemotherapy, HER2-negative/hormone receptor (HR)-negative or endocrine resistant HR-positive; measurable per RECIST 1.1; accessible to tumor biopsy; no bone metastases-only disease, no major organ dysfunction) were subjected to tumor biopsy for genomic analysis (CGH arrays, Affymetrix Cytoscan; NGS, Ion Torrent PGM, AmpliSeq, panel of around 50 genes). Actionable GA were identified and corresponding targeted therapies were proposed by a multidisciplinary tumor board (MTB). Patients received cytotoxic-based treatment at physician's choice and those with stable or responding disease after 6 to 8 cycles (or at least 4 if stopped for toxicity reason) and targetable GA, were offered randomization between targeted therapy or chemotherapy maintenance until progression or intolerance (main study). Since January 2016, an amendment was made to propose to patients without targetable alteration a randomization between anti-PD-L1 (MEDI4736) or standard chemotherapy maintenance (substudy).

Results Between March 2014 and May 2016, 457 patients have been enrolled at 21 centers. Genomic analyses could not be obtained in 107 cases (23%) due to either biopsy failure (n= 40; 9%) or low cellularity (n=67; 14%). Of the 307 patients reviewed by the MTB, 197 (64%) had an actionable GA, including *PIK3CA-PIK3CB-PIK3R1* (n=51), *FGF4* or *FGFR1/2* (n= 42), *BRCA1/2* (n=15), *AKT1/2/3* (n=13), *BRAF/KRAS/NRAS* (n=13), *HER2/3* (n=10), *NF1-FRS2* (n=10), *MTOR-RPTOR-TSC2* (n=8), *PTEN* (n=7), *STK11* (n=7), *IGF1R* (n=7), *EGFR* (n=5). Therapeutic proposals by MTB included AZD5363 (n=71), AZD4547 (n=42), AZD2014 (n=23), selumetinib (n=23), olaparib (n=16), AZD8931 (n=15), vandetanib (n=5), bicalutamide (n=2). In an exploratory analysis involving 157 patients, the rate of targeted therapy proposal by MTB markedly differed between triple-negative patients (TNBC; 24 of 48, 50%) and HER2-negative/HR-positive patients (92 of 109, 84%; p=6.14. 10⁻⁶, Chi-2 test). At the time of the analysis, 85 patients have been randomized (main study, 68; substudy, 17). Causes of randomization failure (n=108) included disease progression (n=45) or death (n=25), non-eligibility criteria (n=27), patient/physician's decision (n=11).

Conclusion A large number of patients had identified targetable GA. Of note, the rate of targeted therapeutic proposal was significantly lower in TNBC than in HER2-negative/HR-positive patients. Rapidly progressing disease may impede ultimate randomization.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD2-01

Title: Clinically dormant ER+ breast tumors exhibit AMPK activation

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Body: While adjuvant anti-estrogen therapy has shown immense clinical benefit for patients with estrogen receptor-positive (ER+) breast cancer, >30% of patients experience cancer recurrence within 15 years of initial diagnosis. Anti-estrogen therapies inhibit ER activity either directly (tamoxifen, fulvestrant) or by reducing systemic estrogen levels (aromatase inhibitors, AIs). Extending adjuvant anti-estrogen therapy from 5 to 10 years further prevents recurrence; however, with both 5- and 10-year treatment regimens, a large proportion of patients who relapse do so after cessation of therapy (“late recurrence”). Additionally, disseminated tumor cells in bone marrow have been found after 4 years of adjuvant anti-estrogen therapy in “disease-free” patients. These data collectively indicate that anti-estrogens elicit clinical benefit as adjuvant therapies, in part, by maintaining a population of residual micrometastatic cancer cells in a “clinically dormant” state (i.e., undetectable by routine clinical methods). Understanding how such dormant cancer cells survive will enable the development of more effective adjuvant therapies. We developed several luciferase-labeled xenograft models of ER+ breast cancer that recapitulate clinical dormancy *in vivo*. Low systemic levels of estrogen in mice can be further suppressed by ovariectomy, mimicking the effects of AI-induced estrogen deprivation seen in patients. In ovariectomized mice, palpable tumors form upon 17 β -estradiol supplementation, but quickly regress upon estrogen withdrawal. While regressed tumors become non-palpable within 2 wk, a small proportion of cancer cells survive these estrogen-deprived conditions for >4 months in a clinically dormant, growth-suppressed state. This estrogen deprivation-induced clinically dormant cell population retains tumorigenic potential, as 17 β -estradiol retreatment induces tumor recurrence. RNA expression profiling revealed AMPK alpha 2 as one of the most highly expressed genes in clinically dormant residual tumor cells compared to acutely estrogen-withdrawn tumors. Increased AMPK kinase activity was confirmed through immunohistochemical analysis of phospho-ACC, and AMPK substrate. AMPK activation using glucose deprivation or the anti-diabetes drug metformin promoted estrogen-independent survival and growth of ER+ breast cancer cells *in vitro*. Metformin treatment may also slow estrogen withdrawal-induced tumor regression and promote tumor cell survival in ER+ breast cancer xenografts. As a cellular energy sensor, AMPK has been shown to promote autophagy, a process linked with anti-estrogen resistance. Immunofluorescent staining of estrogen deprivation-induced clinically dormant residual tumor cells revealed decreased levels of the autophagy marker p62 compared to 17 β -estradiol-driven tumors. Early data suggest that inhibition of autophagy with hydroxychloroquine abrogates cell survival conferred by metformin in estrogen-depleted conditions. Thus, AMPK may be promoting the survival of ER+ breast cancer cells in estrogen-deprived conditions by increasing autophagic flux. These data have implications for the interpretation of data from ongoing clinical studies testing metformin for the treatment of cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD2-02

Title: SFX-01 targets Wnt signalling to inhibit stem-like cells in breast cancer patient-derived xenograft tumours

Howell SJ J, Simoes BM M, Alferez D, Eyre R, Spence K, Santiago-Gomez A, Sarmiento-Castro A, Tanaka I, Howat D and Clarke RB B. University of Manchester; The Christie NHS Foundation Trust and Evgen Pharma Ltd.

Body: Background: SFX-01 is a novel therapeutic comprising synthetic sulforaphane (SFN) stabilised within a-cyclodextrin. Breast cancer stem-like cells (CSCs) have been identified in all molecular subtypes and are likely drivers of breast cancer metastasis and treatment resistance. We recently established that CSC activity in ER+ BC, represent a source of therapeutic resistance (Simões et al, Cell Reports, 2015).

Material and methods: We investigated SFX-01 effects on breast CSC activity using mammosphere formation efficiency (MFE) and aldehyde dehydrogenase (ALDH) activity using the ALDEFLUOR assay in patient samples and patient-derived xenograft (PDX) tumours. Cells from primary (n=12) and metastatic (n=15) samples were treated with SFX-01 (5 µM) or vehicle control. Using a 2 or 8 week *in vivo* treatment, early (HBCx34) and metastatic (BB3RC31) ER+ PDX tumours were treated with SFX-01 (300mg/Kg/day) alone or in combination with tamoxifen (TAM, 10 mg/kg/day) or fulvestrant (FULV, 200 mg/kg/week). Tumours were dissociated and MFE and ALDH activity assessed.

Results: SFX-01 *in vitro* reduced MFE of both primary (0.19%±0.02 vs control 0.52%±0.06: p<0.001) and metastatic patient samples (0.43±0.04 vs control 0.93%±0.07: p<0.001). SFX-01 treatment *in vivo* for 2 weeks reduced MFE of HBCx34 (0.35%±0.03 vs control 0.64%±0.09; p<0.01) and BB3RC31 (0.78%±0.04 vs control 0.89%±0.06: p<0.05) and also ALDH activity of HBCx34 (3%±0.6 vs control 6.3%±0.4: p<0.01) and BB3RC31 (1%±0.2 vs control 3%±0.6: p<0.05). TAM and FULV increased MFE and ALDH activity after 2 weeks of treatment *in vivo*, which was abrogated by combination with SFX-01; for example HBCx34 MFE with TAM alone: 0.81%±0.07 vs TAM+SFX-01: 0.34%±0.02 (p<0.01) and ALDH+ with TAM alone 10%±0.4 vs TAM+SFX 4.2%±0.4 (p<0.01). TAM+SFX-01 suppressed tumour growth at 8 weeks vs TAM alone in HBCx34 but not BB3RC31. FULV treatment maintained tumour growth suppression at 8 weeks and no additive effect was seen with SFX-01, although MFE and ALDH activity were suppressed. Mechanistically, SFX-01 potently inhibited the canonical Wnt pathway in MCF-7 cells and their endocrine-resistant derivatives and we are currently exploring SFX-01 activity on other CSC regulatory pathways. SFX-01 has been shown to be well tolerated in SAD and MAD studies in normal volunteers and clinical studies designed to test tolerability and efficacy in combination with the three major classes of endocrine therapy (AI, TAM and FULV) in advanced BC will begin in Q4 2016.

Conclusions: Our data demonstrate the potential of SFX-01 for clinically meaningful improvements to endocrine therapy in ER+ breast cancer by reversing CSC mediated resistance.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD2-03

Title: Recurrent functionally diverse in-frame *ESR1* gene fusions drive endocrine resistance in breast cancer

Lei JT T, Shao J, Zhang J, Iglesia M, Cao J, Chan DW W, He X, Kosaka Y, Schmidt C, Matsunuma R, Haricharan S, Crowder R, Hoog J, Phommaly C, Goncalves R, Ramalho S, Lai W-C, Hampton O, Rogers A, Tobias E, Parikh P, Davies S, Ma C, Suman V, Hunt K, Watson M, Hoadley KA A, Thompson A, Chen X, Perou CM M, Creighton CJ J, Maher C and Ellis MJ J. Baylor College of Medicine, Houston, TX; Washington University School of Medicine, St. Louis, MO; University of North Carolina, Chapel Hill, NC; Kitasato University School of Medicine, Minato, Japan; University of Sao Paulo School of Medicine, Sao Paulo, Brazil; State University of Campinas, Sao Paulo, Brazil; Mayo Clinic, Rochester, MN and MD Anderson Cancer Center, Houston, TX.

Body: Background. We previously reported an alternative *ESR1* somatic gain-of-function chromosomal translocation event in a patient presenting with aggressive, endocrine therapy resistant estrogen receptor (ER) positive disease, producing an in-frame fusion gene consisting of N-terminal *ESR1* and the C-terminus of the Hippo pathway coactivator *YAP1* (*ESR1-YAP1*). We recently identified another *ESR1* fusion through RNA sequencing (RNA-seq) in advanced stage ER+ disease from a chest wall recurrence in a male patient that was refractory to multiple lines of treatment. Two examples of fusions discovered in primary breast cancer samples include *ESR1* fused in-frame to C-terminal sequences from *NOP2* (*ESR1-NOP2*), identified in a resistant cohort from a RNA-seq screen focused on 81 primary breast cancers from aromatase inhibitor clinical trials, and a second *ESR1* fusion, fused in-frame to the entire coding sequence of *POLH* (*ESR1-POLH*), that was identified from RNA-seq analysis of 728 Cancer Genome Atlas breast samples. This current study extends our previous characterization of *ESR1-YAP1* by comparing functional and pharmacological properties of these three additional *ESR1* gene fusion events of both early stage and advanced breast cancers.

Methods. *In vitro* and *in vivo* experiments were conducted to test *ESR1* fusions to induce therapeutic resistance, and metastasis. The transcriptional and binding properties of each fusion was also examined. Pharmacological inhibition with Palbociclib, a cyclin-dependent kinase 4/6 inhibitor, was utilized to assess drug sensitivity in *ESR1* fusion containing breast cancer cells and in a patient derived xenograft (PDX) model expressing *ESR1-YAP1* (WHIM18).

Results. The *YAP1* and *PCDH11x* fusions conferred estrogen-independent and fulvestrant-resistant growth. Immunohistochemistry revealed significantly higher numbers of ER+ cells in lungs of mice xenografted with T47D cells expressing the *YAP1* and *PCDH11x* fusions compared to YFP control, *NOP2* and *POLH* fusions. Results from ChIP-seq and microarray studies suggest that these two fusions promote proliferation and metastasis through genomic action by binding estrogen response elements (ERE) and subsequent gene activation. We thereby define these fusions as “canonical” fusions compared to “non-canonical” *NOP2* and *POLH* fusions, which demonstrated dramatically decreased genomic binding ability. The non-canonical fusions induced genes associated with basal-like breast cancer and promoted HER2, EGFR, and MAPK gene expression signatures in contrast to genes associated with cell cycle/proliferation induced by canonical fusions. The proliferative ability of canonical fusion-containing ER+ cells was inhibited by Palbociclib in a dose-dependent manner. *In vivo* WHIM18 tumors in mice fed with Palbociclib-containing chow demonstrated significantly reduced tumor volume, growth rate, and weight compared to tumors in mice on control chow.

Conclusions. In-frame ERE activating canonical fusions occur in end-stage drug resistant advanced breast cancer and can be added to *ESR1* point mutations as a class of recurrent somatic mutation that may cause acquired resistance. Growth induced by these fusions can be antagonized by Palbociclib and is potentially clinically helpful.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD2-04

Title: FOXA1 induces a pro-metastatic secretome through ER-dependent and independent transcriptional reprogramming in endocrine-resistant breast cancer

Fu X, Pereira R, Zhao D, Jung SY Yun, Jeselsohn R, Creighton CJ J, Shea M, Nardone A, Angelis CD De, Tsimelzon A, Wang T, Gutierrez C, Huang S, Edwards DP P, Rimawi MF F, Hilsenbeck SG G, Brown M, Chen K, Osborne CK Kent and Schiff R. Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX; Dan L. Duncan Comprehensive Cancer Center, Baylor College of Medicine, Houston, TX; Baylor College of Medicine, Houston, TX; Baylor College of Medicine, Houston, TX; Baylor College of Medicine, Houston, TX; Baylor College of Medicine, Houston, TX; Center for Cardiovascular Regeneration, The Methodist Hospital Research Institute, Houston, TX and Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

Body: Background: Metastasis in ER-positive (+) breast cancer (BC) occurring years to decades after initial diagnosis presents a daunting challenge for clinical care and preclinical research due to limited known key players and experimental models. FOXA1 is a pioneer factor for ER-chromatin binding and function, and is highly expressed in ER+ BC metastases, yet the underlying mechanism is unclear. Tumor-secreted proteins play a crucial role in the reciprocal interplay between cancer cells and host microenvironmental factors at both primary and secondary sites. We hypothesized that high FOXA1 provokes an ER-dependent transcriptional program that includes a unique pro-tumorigenic secretome essential for promoting ER+ BC metastasis. **Methods:** A lentiviral doxycycline (Dox)-inducible FOXA1 overexpression vector and a dual luciferase/GFP (LG) tracking vector were integrated to construct a stable MCF7-LG/FOXA1 cell model. Ovariectomized nude mice bearing MCF7-LG/FOXA1 xenografts in the presence of exogenous estrogen (E2) were randomized to \pm Dox, each with continued E2, E2 deprivation (ED), or tamoxifen (Tam). Survival surgery removing the therapy-naïve (E2 arm) and relapsed (ED/Tam arms) tumors was performed when tumors reached ~ 1000 mm³. All mice then received ED/Tam 'adjuvant' therapy, with longitudinal luminescence imaging to monitor local/distant recurrences. Mice were or will be euthanized at the ethical end-point. Integrative bioinformatics was performed using RNA-seq and FOXA1/ER ChIP-seq data from our preclinical models to identify secretome targets for functional intervention. Times to tumor regression (TTR) and progression (TTP) were defined by when the tumor reached half or twice the volume at randomization. **Results:** Median (m) TTR was achieved in ED (31/34 days, $-/+Dox$, $P = 0.184$) but not in Tam groups — Tam delayed tumor growth but failed to prevent progression in all mice with mTTP of 94/93 days ($-/+Dox$, $P = 0.517$). Despite no difference in mTTP at Tam $-/+Dox$, a quarter of +Dox tumors (3/12) had volume doubled by day 11. No metastases were observed by imaging in any of the mice before surgery ('neoadjuvant' setting). Local relapse and lymph-node/lung metastases were detected after surgery ('adjuvant' setting). At day 90 in the adjuvant Tam group with previously relapsed tumors, +Dox mice succumbed to metastasis more often than -Dox mice (7/8 vs. 3/10, $P = 0.023$). Compared to the adjuvant Tam+Dox mice with previous therapy-naïve tumors, the Tam+Dox with previously relapsed tumors showed higher distant metastasis rate (7/8 vs. 5/14, $P = 0.026$). Analysis of the ED setting is pending due to late recurrence. Data integration and functional study revealed a set of cytokines, growth factors, and extracellular matrix components (including IL-8, CTGF, and LOX), regulated by FOXA1 often in conjunction with ER, that are highly involved in FOXA1-induced metastasis. Global secretome profiling by mass spectrometry and target validation are ongoing. **Conclusions:** FOXA1 overexpression increases metastatic potential in ER+ BC. We established a pertinent metastatic xenograft mouse model to characterize a pro-metastatic secretome with diagnostic and therapeutic potential for treating metastatic ER+ BC.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD2-05

Title: Inhibition of mutant HER2 results in synthetic lethality when combined with ER antagonists in ER+/HER2 mutant human breast cancer cells

Croessmann S, Zabransky DJ J, Cutler, Jr. RE E, Lalani AS S, Park BH H and Arteaga CL L. Vanderbilt University Medical Center, Nashville, TN; Johns Hopkins School of Medicine, Baltimore, MD and Puma Biotechnology, Inc., Los Angeles, CA.

Body: Background: Human epidermal growth factor receptor 2 (ERBB2 or commonly known as HER2) missense mutations have been reported in 2-4% of breast cancers and occur primarily in the absence of *HER2* gene amplification. Based on TCGA, approximately 60% of these tumors are hormone-dependent and express estrogen receptor (ER) α . Among ER+ breast cancers with HER2 missense mutations, more than 80% are in the HER2 kinase domain. We examined herein whether ER+/HER2 mutant breast cancer cells are resistant to anti-estrogen therapies and, thus, whether they should be treated with combined ER and HER2 inhibitors.

Methods: Three common HER2 activating mutations (G309A, L755S, V777L) and wild type (WT) *HER2* were incorporated into ER+ MCF7 cells using AAV-mediated homologous recombination. The isogenic incorporation of a heterozygous mutation more accurately represents primary human tumors as compared to transfection and overexpression of exogenous vectors. We examined cell viability and ER transcriptional activity, using an ERE-luciferase reporter, in response to estrogen deprivation and treatment with fulvestrant (a selective ER downregulator) and neratinib (an irreversible, pan-HER tyrosine kinase inhibitor), either alone or in combination. Signaling downstream mutant HER2 was examined by immunoblot analysis. *In vivo* anti-tumor efficacy of fulvestrant \pm neratinib is currently being assessed in ovariectomized athymic mice bearing MCF7/HER2^{V777L} xenografts.

Results: MCF7 cells containing HER2 kinase missense mutations (L755S and V777L), but not cells with HER2^{WT} or an extracellular domain mutation (G309A), were able to proliferate exponentially in estrogen-free medium. MCF7/HER2^{L755S} and MCF7/HER2^{V777L} were also resistant to 1 mM fulvestrant, despite fulvestrant's ability to downregulate ER in these cells. Additionally, MCF7/HER2^{L755S} and MCF7/HER2^{V777L} showed increased levels of pERK and p70S6K. Treatment with 200 nM neratinib potently inhibited growth of MCF7/HER2^{L755S} and MCF7/HER2^{V777L} in estrogen-free conditions and resensitized them to fulvestrant while partially downregulating HER2 levels. Addition of 1 nM estradiol markedly rescued all three HER2 mutant cells from neratinib-induced cell death suggesting that the inhibition of both ER and mutant HER2 is required for tumor cell apoptosis. Using ERE-luc reporter assays, neratinib did not inhibit basal or estrogen-induced ER transcriptional activity or ER α Ser118 phosphorylation, thus not supporting HER2 mutation-to-ER crosstalk in these genetically engineered cells. This result also suggests that the ER and HER2 mutant pathways can operate independently and it is the dual pathway inhibition that results in synthetic lethality.

Conclusions: These data suggest that, in ER+ breast cancers and similar to HER2 gene amplification, HER2 kinase domain mutations induce resistance to antiestrogen therapies. Therefore, we propose simultaneous therapeutic targeting of both ER and HER2 signaling pathways is required for maximal inhibition of ER+ breast cancers also harboring HER2 activating mutations, as is currently being investigated in the phase II SUMMIT trial (NCT01953926).

2016 San Antonio Breast Cancer Symposium

Publication Number: PD2-06

Title: Mismatch repair deficiency induces endocrine therapy resistance in breast cancer

Haricharan S, Schmelz J, Schmidt C, Singh P, Holloway K, Anurag M, Suman V, Olson JA A, Hunt K, Bainbridge MN N and Ellis MJ J. Baylor College of Medicine; Mayo Clinic; University of Maryland School of Medicine and UT MD Anderson Cancer Center.

Body: Estrogen receptor positive (ER⁺) breast cancer accounts for the majority of breast cancers diagnosed worldwide but fortunately, outcomes are markedly improved by pharmacological interventions that interrupt ER function. Unfortunately, suppression of relapse risk from early stage disease with endocrine therapy (anti-estrogens or aromatase inhibitors) is only ~50%, and for advanced disease, endocrine therapy resistance is almost inevitable. While many mechanisms for intrinsic and acquired endocrine resistance have been explored, links between defects in DNA repair, the fundamental drivers of cancer pathogenesis, and endocrine therapy resistance have been understudied. Here we link mismatch repair (MMR) deficiency to poor clinical outcomes in ER⁺ breast cancer using whole exome DNA sequencing data and mRNA expression analysis. We subsequently demonstrate that MMR deficiency bypasses ER dependent cell cycle regulation through disruption of Chk2/p21-mediated feedback inhibition of CDK4 in breast cancer cell lines and tumor samples, as well as through correlations with human clinical data. We also show that pharmacological targeting of CDK4 significantly inhibits growth of MMR-deficient ER⁺ breast cancer cells in vitro and in vivo. This mechanism provides a new explanation for why endocrine therapy resistant ER⁺ breast cancers can respond to CDK inhibition and suggests that primary tumors exhibiting MMR deficiency are good candidates for adjuvant CDK4 inhibitor treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD2-07

Title: 10-year follow-up and biomarker discovery for adjuvant endocrine therapy; results of the TEAM trial

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Body: Optimal endocrine therapy for postmenopausal, hormone-receptor positive (HR+) early breast cancer remains a point of discussion. The Tamoxifen Exemestane Adjuvant Multinational (TEAM) phase III trial showed no significant differences for disease free survival (DFS) and overall survival (OS) at 5 years between exemestane monotherapy and sequential treatment (tamoxifen followed by exemestane). We now report disease related outcomes at 10 years of follow-up (FU), and an explorative analysis to assess the predictive value of clinicopathological and immune-related biomarkers.

In the TEAM trial, postmenopausal women with HR+ positive early breast cancer were randomly assigned to exemestane alone or sequential therapy. For this analysis, TEAM patients from countries that completed 10 years of FU were included. The primary endpoint was DFS at ten years, analyzed by intention to treat. Secondary outcomes were OS and cumulative incidence of relapse. An explorative per protocol analysis for relapse free survival (RFS) was performed to identify predictive pathological and immunological biomarkers, including centrally determined ER (ER-poor 0-6 vs ER-rich 7-8) and PR (0-4 vs 5-8) Allred scores, and the immunological markers CD8, FoxP3, classical HLA class 1 and HLA-G which were described earlier (Engels et al, Breast Cancer Treat Res, 2015).

In total, 6120 patients were eligible for the current analysis, 3075 patients with exemestane monotherapy and 3045 patients randomized to sequential treatment. Median follow up was 9.83 years. DFS was 66.8% in the exemestane group and 66.8% in the sequential group (hazard ratio (HR) 0.96, 95% CI 0.88-1.05, $p=0.389$). OS was 74% in the exemestane, and 73% in the sequential group, respectively (HR 0.98, 95% CI 0.89-1.09, $p=0.737$). The cumulative incidence of relapse was 20% and 22% in the exemestane and sequential groups, respectively (HR 0.88, 95% CI 0.79-0.99, $p=0.031$).

In the explorative per protocol analysis ($n=4041$), Allred score were available for 2996 patients; immunological markers for 1754 patients. Patients with above median numbers of FoxP3-positive T-cells showed a benefit of exemestane monotherapy for RFS (HR 0.56, 95% CI 0.42-0.75, $p<0.001$) in contrast to patients with low numbers of FoxP3-positive cells (HR 1.0, 95% CI 0.77-1.32, $p=0.97$, p -value for interaction 0.004). A high tumor differentiation grade was associated with more benefit for exemestane monotherapy (grade 1/2 HR 0.78, 95% CI 0.65-0.94, $p=0.01$, grade 3/4 HR 0.61, 95% CI 0.49-0.75, $p<0.001$), with a borderline significant interaction ($p=0.07$). ER Allred score showed a borderline significant treatment by marker effect interaction (ER-rich HR 0.69 (95% CI 0.58-0.81, $p<0.001$); ER-poor HR 0.94 (95% CI 0.65-1.34, $p=0.71$, p for interaction 0.12).

After ten years of follow up, both exemestane monotherapy and sequential therapy remain appropriate options for postmenopausal HR+ early breast cancer patients. Interestingly, the number of regulatory T-cells was a predictive factor for the benefit of exemestane monotherapy, which implies a role of the local immune system in endocrine therapy. Furthermore, data suggested that patients with a higher differentiation grade or ER-rich tumor derive more benefit from exemestane monotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD2-08

Title: Neratinib + fulvestrant in *ERBB2*-mutant, HER2–non-amplified, estrogen receptor (ER)-positive, metastatic breast cancer (MBC): Preliminary analysis from the phase II SUMMIT trial

Hyman D, Piha-Paul S, Saura C, Arteaga C, Mayer I, Shapiro G, Loi S, Lalani A, Xu F, Cutler R, Butturini A, Bryce R, Meric-Bernstam F, Baselga J and Solit D. Memorial Sloan Kettering Cancer Center; MD Anderson Cancer Center; Vall d'Hebrón University Hospital, Vall d'Hebrón Institute of Oncology (VHIO); Vanderbilt-Ingram Cancer Center; Dana Farber Cancer Institute; Peter MacCallum Cancer Centre and Puma Biotechnology Inc.

Body: Background: Somatic mutations in *ERBB2* are a new class of oncogenic drivers in HER2–non amplified MBC. Neratinib is an irreversible pan-HER tyrosine kinase inhibitor that inhibits the growth of *ERBB2*-mutant breast tumors in preclinical models and has encouraging single-agent clinical activity in patients (pts) with *ERBB2*-mutant, HER2–non amplified MBC. Bi-directional signaling between HER2 and ER may limit the effectiveness of endocrine and HER2 directed therapy, if each is given alone, in ER+ MBC with *ERBB2* amplifications/mutations. Preclinical data suggest that dual blockade of ER and HER2 signaling results in enhanced anti-tumor activity in ER+ HER2+ MBC. SUMMIT, a multicenter multi-histology phase II 'basket' trial, is investigating the efficacy of neratinib monotherapy (in ER+ and ER– pts) and neratinib + fulvestrant (ER+ pts only) in *ERBB2*-mutant MBC.

Methods: MBC pts with *ERBB2* mutations documented by local testing were eligible and received oral neratinib 240 mg qd. Pts with ER+ MBC received fulvestrant 500 mg, a selective ER degrader, in addition to neratinib on d1 & 15 of month 1 then on d1 q4w. Patients received high dose loperamide prophylaxis during cycle 1. Primary endpoint is objective response rate (ORR) at 8w, defined using RECIST 1.1 and/or modified PERCIST assessments. Secondary endpoints include ORR, clinical benefit rate (CBR), progression free survival (PFS), and safety. Mutation profiling and central confirmation of *ERBB2* mutation(s) from available fresh or archival tumor tissues and plasma DNA were performed retrospectively by next-generation sequencing (MSK-IMPACT). Clinicaltrials.gov: NCT01953926.

Results: As of 23 Sep 2016, 35 efficacy-evaluable *ERBB2*-mutant MBC pts received neratinib, either as monotherapy (n=24) or in combination with fulvestrant (n=11). Efficacy findings are shown in the table.

	Neratinib monotherapy (n=24)	Neratinib + fulvestrant (n=11)
Best Overall Response (confirmed and unconfirmed), n (%) [95% CI]	8 (33.3) [15.6–55.3]	6 (54.5) [23.4–83.3]
CR	3 (12.5)	2 (18.2)
PR	5 (20.8)	4 (36.4) ^a
ORR at 8 weeks, n (%) [95% CI]	8 (33.3) [15.6–55.3]	5 (45.5) [16.7–76.6]
CR	2 (8.3)	2 (18.2)
PR	6 (25.0)	3 (27.3)
ORR confirmed, n (%) [95% CI]	5 (20.8) [7.1–42.2]	2 (18.2) ^b [2.3–51.8]
CR	3 (12.5)	1 (9.1)
PR	2 (8.3)	1 (9.1)
CBR, n (%) [95% CI]	10 (41.7) [22.1–63.4]	6 (54.5) [23.4–83.3]

CR, complete response; PR, partial response.

^aThere was 1 pt with PR at week 16; ^bAt time of data cut-off 4 pts are still on treatment.

The overall safety profile of neratinib + fulvestrant was similar to that previously reported with neratinib monotherapy. Grade 3 diarrhea rate was 24% with neratinib monotherapy and 18% with neratinib + fulvestrant.

Conclusions: Encouraging clinical activity has been observed with neratinib + fulvestrant in heavily pretreated pts with *ERBB2*-mutant, ER+ MBC. Clinical efficacy in the ER+ MBC cohort met pre-specified efficacy requirements; a confirmatory trial of neratinib + fulvestrant for targeting *ERBB2* mutations in ER+ MBC is warranted. The safety profile of neratinib was acceptable and diarrhea was manageable with loperamide prophylaxis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-01

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-02

Title: The predictive value of sentinel node biopsy (SNB) in early breast cancer after neo-adjuvant chemotherapy (NACT): A prospective study

Huizing M, Najim O, Dockx Y, Huyghe I, Van den Wyngaert T, van Goethem M, Verslegers I, Papadimitriou K, Altintas S, Baldewijns M, Trinh B, van Dam P and Tjalma W. Multidisciplinary Breast Clinic, Antwerp University Hospital – University of Antwerp, Edegem, Belgium.

Body: Background

SNB has replaced axillary lymph node dissection (ALND) in those patients (pts) with clinically node negative axilla. This has reduced the morbidity, in particular lymphedema considerable. SLN after NACT is feasible but not accurate in clinically node positive (cN1-3) pts (false negative rate around 10%). Therefore, continuous efforts have to be made in randomized prospective studies to improve the detection rate of SNB in order to avoid the morbidity of ALND. The purpose of this study is to determine the negative predictive value of the sentinel node in breast cancer after NACT.

Method

A single institution prospective study regarding the negative predictive value of the sentinel node in breast cancer after NACT was conducted in the Multidisciplinary Breast Clinic of the Antwerp University Hospital from 29/03/2010 until 12-2015 (Study number: B30020108368). Inclusion criteria for study participation were: breast cancer, age above 18 years, female, tumor stages T2-T4 N0-3 or T1N1-N3. All pts were staged by a mammography, ultrasound of the axilla, MRI of the breast, ¹⁸F-fluoro-2-deoxy-glucose (¹⁸F-FDG) positron emission tomography (PET-CT) scan and bone scintigraphy. They received NACT consisting of 12 cycles of Paclitaxel or 4 cycles of Docetaxel followed by dose dense doxorubicin or epirubicin/cyclofosfamide or vice versa as a standard initial treatment. After 6 weeks a ¹⁸F-FDG PET-CT scan was performed for early tumor response evaluation. At the day of operation, all the pts had a preoperative injecting with a ^{99m}Tc-labelled nanocolloid in the peri-areolar region. A gamma detector was used to localize the SLN(s). All SLN(s) were removed and a complete ALND was performed.

Results

A total of 150 pts were enrolled in our study of which 129 were eligible for analysis. 53 pts had a positive SLN of which 32 have a positive axillary lymph nodes (ALN) (PPV 60%); 76 pts has a negative SLN of which 6 had a positive ALN (NPV 92%). The sensitivity is 84% and the specificity 76% with a false omission rate (FOR) of 8%. 45 pts had an initial clinical N0 (cN0 is defined as clinical negative and no suspect lymph nodes on ultrasound, on MRI breast and ¹⁸F-FDG-PET CT scan). 45 pts had negative SLN, with no ALN and 2 pts had a positive SNL of which 1 pts had axillary involvement (NPV 100%). The FOR of cN1: 5%, cN2: 37%, cN3 33%. A total of 22 pts out of 84 pts (26%) of which 15/49 cN1 (30%), 6/23 (26%) cN2, 1/12 (8%) have after 6 weeks of chemotherapy, ¹⁸F-FDG normalization on ¹⁸F-FDG PET-CT scan. A total of 17 pts had a negative SLN and ALN. The FOR was 0%

Conclusion

SNB after NACT in case of cN0 is very reliable with high NPV and low FOR. In case of ¹⁸F-FDG-PET CT normalization after 6 weeks of chemotherapy and a negative SLN, no ALND has to be performed.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-03

Title: What to do with non-visualized sentinel nodes; to dissect or not to dissect the axilla?

Verheuve NC C, Voogd AC C, Tjan-Heijnen VCG CG and Roumen RMH MH. Máxima Medical Center, Veldhoven, Netherlands; School for Oncology and Developmental Biology (GROW) Maastricht University Medical Center, Maastricht, Netherlands; School for Oncology and Developmental Biology (GROW) Maastricht University Medical Center, Maastricht, Netherlands and Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, Netherlands.

Body: Background Both in the literature and in international guidelines evidence is scarce on clinicopathological characteristics and axillary treatment recommendations in patients with a non-visualized sentinel node (nvSN) during the sentinel lymph node (SLN) procedure. Therefore, this study aims to evaluate the prevalence of nvSN in a Dutch population of breast cancer patients and to compare their characteristics and prognosis with patients in whom the SLN could be visualized. Moreover, we have distributed a questionnaire among certified oncological surgeons in the Netherlands in order to determine their routine regarding the axillary treatment after a nvSN.

Methods A retrospective population based study was performed including patients diagnosed with invasive breast cancer in the Netherlands between January 2000 and December 2013. Patients were included if they had no clinically palpable lymphadenopathy (cN0) or clinically apparent metastases (cM0). Patients receiving neo-adjuvant systemic treatment, patients with palpable axillary nodes and patients who did not undergo a SLN procedure were excluded.

Also, a questionnaire containing 10 questions regarding clinical routine during the sentinel node procedure and axillary treatment of nvSN patients was distributed among 150 oncological (breast) surgeons.

Results Of the 101,289 patients who fulfilled the inclusion criteria, 2545 (2.5%) had a nvSN. Univariate and multivariate analyses show that patients with a nvSN were older ($p < 0.001$), were more often diagnosed in the years 2000-2005 ($p < 0.001$), had a larger tumor ($p = 0.003$) with more often a mastectomy ($p = 0.02$) and were more likely to have ≥ 3 positive lymph nodes ($p < 0.001$) compared to patients in whom the SLN could be visualized. However, adjusted survival analyses showed a borderline not-significant survival difference between these groups (HR=1.23, 95%CI=0.99-1.28). Of the 2545 patients with a nvSN, 2127 (84%) patients underwent an axillary lymph node dissection (ALND). Multivariate analyses show that patients receiving an ALND were more often diagnosed in the years 2000-2005, had a larger tumor and more often received adjuvant systemic therapy with both hormonal and chemotherapy. Adjusted survival analyses showed no statistically significant association between ALND and survival (HR=0.89, 95%CI=0.92-1.27).

The questionnaire was completed by 122 (24%) oncological (breast) surgeons. It showed that 39% of the respondents estimated the prevalence of a nvSN to be 1-2%. Most surgeons are currently more reserved to perform an ALND than before the Z0011 trial, depending on various clinicopathological characteristics; 23 respondents answered to opt for an alternative axillary treatment option.

Conclusion NvSN patients had worse disease characteristics compared to patients in whom the sentinel node could be visualized, though an ALND was not associated with a better survival. The results of the questionnaire show that surgeons are more reluctant to perform an ALND in case of a nvSN, especially after publication of the Z0011 trial, and that they would like the guideline to be revised and clarified regarding the axillary treatment in case of a nvSN.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-04

Title: Use of axillary ultrasound impacts outcome of node positive breast cancer patients

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Body: Background The Z0011 trial initiated a paradigm shift in the treatment of axillary node positive breast cancer patients. Treatment strategy, however, starts with the information gathered by the diagnostic axillary work up. This can either be done by sentinel lymph node biopsy (SLNB) or ultrasound guided lymph node biopsy (UGLNB). We examined whether there are relevant clinical and prognostic differences between patients found node positive by these two diagnostic selection processes.

Methods Patients diagnosed with invasive breast cancer in the Netherlands between January 2000 and December 2013 were studied. Patients with no clinically palpable lymphadenopathy (cN0) and node-positive disease after an axillary lymph node dissection (ALND) were included. Patients with stage IV breast cancer, with clinical stage T₃-T₄ breast tumor according to the TNM-classification, those treated within the neo-adjuvant setting, patients with palpable axillary nodes (cN₁) and patients who did not undergo an ALND were excluded.

Results A total of 14,730 patients fulfilled the inclusion criteria, of whom 9,448 were included in the SLNB group and 5,282 in the UGLNB group. Patients in the UGLNB group were older at diagnosis ($p < 0.001$), had larger tumors ($p < 0.001$), a higher tumor grade ($p = 0.001$), and were more likely to have a negative hormonal receptor status ($p < 0.001$) and to undergo a mastectomy ($p < 0.001$). Patients in the UGLNB group were also more likely to have ≥ 3 positive axillary lymph nodes ($p < 0.001$) and, after adjustment for these differences, had a worse overall survival (HR=1.64; 95% CI=1.53-1.75) compared to the node-positive patients in the SLNB group.

Conclusion Our multicenter study shows that patients with a positive UGLNB have less favorable disease characteristics and a worse prognosis compared to patients with a positive SLNB. The diagnostic selection process plays an important role when axillary treatment strategies are considered. Therefore, we conclude that the conclusions of the Z0011 trial cannot (yet) be applied to patients with a positive UGLNB.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-05

Title: A phase II clinical trial of VST-1001 (dilute fluorescein) in lymphatic mapping and sentinel lymph node localization in clinically node negative breast cancer

Ross MI I, Black DM M, Mittendorf EA A, Porretta JM M, Bedrosian I, Caudle AS S, Hwang RF F, Meric-Bernstam F, Babiera GV V, Brulotte M, Andtbacka RHI HI and Matsen CB B. University of Texas MD Anderson Cancer Center, Houston, TX; Huntsman Cancer Institute, University of Utah, Salt Lake City, UT and Vestan Inc., Salt Lake City, UT.

Body: Background: Combined use of a radiocolloid and a vital blue dye is recommended for accurate lymphatic mapping and sentinel lymph node (SLN) identification in breast cancer. However, vital blue dyes can cause tattooing, skin necrosis and severe allergic reactions. Moreover, the vital blue dyes are only able to detect 70% or less of SLNs in large multi-center trials. Hence, there is an unmet need to develop new lymphatic mapping agents that could potentially replace vital blue dyes. We have previously, in a Phase I trial, reported on the safety of VST-1001 (dilute fluorescein) in SLN identification. Here we report the Phase II data of VST-1001 and direct visualization devices in lymphatic mapping, SLN identification, and safety in clinically node negative breast cancer.

Methods: This prospective Phase II, multi-center, non-randomized, single-arm, open-label, single-dose clinical trial enrolled patients (pts) with DCIS and clinical stage I/II breast cancer eligible for SLN biopsy. All pts had SLN localization with technetium-99m-sulfur colloid (Tc^{99m}SC) and intraoperative lymphatic mapping with 0.1% VST-1001 injected peritumorally, periareolarly, and/or intradermally. SLN radioactivity was identified with a gamma probe, and VST-1001 fluorescence was induced by light emitting diodes and detected as yellowish-green fluorescence in the visible light range with notch filter spectacles. The primary endpoint was the ability of VST-1001 to localize lymph nodes. SLN concordance of Tc^{99m}SC radioactivity and VST-1001 fluorescence, and safety were also assessed.

Results: Eighty-seven women and 2 men with a median age of 60 yrs (range, 37-77) were enrolled. Primary tumor T-stage was: 12.4% T0, 62.9% T1, 23.6% T2, and 1.1% T3. Of the 89 pts, 87 (97.8%) had at least 1 radioactive SLN, and 86 (96.6%) at least 1 fluorescent SLN. Of a total of 198 SLN identified (mean 2.2 SLN/pt), 74.2% were fluorescent and radioactive, 11.6% were radioactive only, 8.6% were fluorescent only, and 5.1% were not radioactive or fluorescent. 82.8% of all SLNs were fluorescent. Twelve (13.5%) pts had microscopic metastatic breast cancer in 14 (7.1%) SLNs. Of the 14 SLNs with metastasis, 12 (85.7%) were both radioactive and fluorescent, 1 (7.1%) fluorescent only and 1 (7.1%) not radioactive or fluorescent and only suspicious on palpation. The fluorescent only SLN was identified in a patient with only 1 SLN and without VST-1001 the metastasis would have been missed. The only adverse event related to VST-1001 was intraoperative grade 2 allergic reaction of the ipsilateral breast in one pt. Intravenous anti-histamines were administered and the erythema resolved.

Conclusions: VST-1001 safely localized lymph nodes in breast cancer. VST-1001 was able to localize lymph nodes that were not radioactive and had a high co-localization concordance with Tc^{99m}SC. VST-1001 also appears to have a higher rate of SLN localization compared to that historically reported for vital blue dyes. In light of these data, VST-1001 may be an alternative SLN localizing agent to be used in conjunction with Tc^{99m}SC in breast cancer pts, eliminating many of adverse events observed when using vital blue dyes without compromising SLN identification.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-06

Title: Patterns of axillary evaluation in older patients (pts) with breast cancer and impact on adjuvant therapy

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Body: Background:

Axillary lymph node status has traditionally been a key factor in informing adjuvant therapy recommendations for pts with breast cancer. With increased emphasis on tumor biology, this information may be less relevant, particularly in older populations where competing comorbidity frequently influences treatment decisions. We examined patterns of axillary surgery in older breast cancer pts and the impact axillary surgery has on treatment receipt.

Methods:

We identified women aged ≥ 65 with Stage I-III invasive breast cancer diagnosed during 2012-2013 from the National Cancer Data Base who did not have clinically positive nodes and underwent cancer-directed surgery. Nodal surgery type and receipt of adjuvant therapies were examined. Multivariable logistic regression was used to examine the associations of axillary surgery receipt with pt, clinical and facility factors.

Results:

Among 69,414 eligible women, 40% were aged 65-70, 42% aged 71-80 and 18% aged >80 . 91% had axillary surgery (67% sentinel lymph node biopsy, 11% axillary lymph node dissection, 13% unspecified axillary surgery), and 24% of pts had pathologically positive nodes. 10% of pts (stage IIB-III) received adjuvant chemotherapy, 81% (hormone receptor positive) received adjuvant hormonal therapy, 67% (breast conservation or stage III postmastectomy) received radiation. In adjusted analyses, increasing age and neoadjuvant hormonal therapy were strongly associated with lower odds of axillary surgery. Region and mastectomy were strongly associated with higher odds of axillary surgery. The table shows variables associated with axillary surgery.

Variable	Adjusted OR (95% CI) for having any axillary surgery*
Age (vs. 65-70)	
71-75	.64 (.58-.71)
76-80	.34 (.31-.37)
>80	.08 (.07-.09)
Diagnosed in 2013 (vs. 2012)	1.08 (1.02-1.15)
Stage (vs. II)	
I	1.25 (1.13-1.38)
III	.73 (.60-.89)
Grade (vs. 1)	
2	1.22 (1.14-1.31)
3	1.24 (1.13-1.37)
HER2 status (vs. positive)	
Negative	.83 (.73-.93)
Tumor size (vs. ≤ 2 cm)	
>2 -5cm	1 (.91-1.11)
>5 cm	.56 (.47-.67)
Comorbidity score (vs. 0)	
1	.85 (.79-.92)

>=2	.62 (.56-.68)
Region (vs. New England)	Range 1.66-2.67 (1.42-3.12)
Case volume (vs. high)	
Low	.82 (.73-.93)
Medium	.98 (.91-1.06)
Insurance (vs. private)	
Uninsured	.69 (.44-1.09)
Medicaid	.67 (.52-.86)
Medicare	.84 (.77-.93)
Median household income (\$) (vs. >46K)	
<30,000	1.06 (.95-1.18)
30,000-34,999	1.11 (1.01-1.21)
35,000-45,999	1.08 (1.01-1.16)
Neoadjuvant hormonal therapy (vs. not)	.49 (.42-.59)
Surgery (vs. BCS)	
Mastectomy, no recon	3.37 (3.09-3.68)
Mastectomy, +recon	2.76 (2.16-3.51)

*Adjusted for table variables plus race, hormone receptor status, and facility type (none significantly associated with axillary surgery)

Axillary surgery and younger age were significantly associated with receipt of adjuvant chemotherapy, radiation, and hormonal therapy.

Conclusion:

Within the NCDB, 91% of pts age ≥ 65 with clinically node-negative breast cancer undergo surgical staging of the axilla, and axillary surgery was associated with adjuvant therapy receipt. The impact of routine node assessment on treatment and outcome has been questioned, and further study in this population of pts is warranted.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-07

Title: Selective elimination of axillary surgery after primary systemic treatment in clinically node-positive breast cancer patients by combining PET/CT and the MARI procedure (marking the axilla with radioactive iodine seeds)

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Body: *Background* The increasing use of primary systemic treatment (PST) for patients with breast cancer enables more breast conserving surgery. In addition, PST converts node-positive into node-negative disease in 20-40% of patients. However, the current guidelines still recommend axillary lymph node dissection (ALND) for clinical node-positive disease (cN+), even if it became node-negative after PST, since false-negative rates of sentinel lymph node biopsy after PST range from 5-30%. Recently, an alternative technique has been introduced to stage the axilla after PST: the MARI-procedure (sensitivity 97%; FNR 7%), in which a tumour-positive lymph node is marked with a radioactive iodine seed before the start of PST and selectively removed after PST. In the present study, we propose a new strategy for treatment of the axilla in cN+ patients by combining results of the pre-PST PET/CT with the post-PST MARI-procedure.

Material and methods All patients who received a MARI-procedure from July 2014 until May 2016 were included. Before the start of PST a PET/CT was performed for axillary staging and the detection of distant metastasis. A radioactive iodine seed was placed in a proven tumour-positive axillary lymph node (MARI-node), after which PST was given according to Dutch national guidelines. At our institute, we have implemented a protocol in which results of the pre-PST PET/CT and the post-PST MARI-procedure determine the type of axillary treatment. Patients with 1-3 positive axillary lymph nodes (ALNs) on PET/CT and a tumour-negative MARI-node receive no further axillary treatment. Patients with ≤ 3 positive ALNs on PET/CT and a tumour-positive MARI-node receive axillary radiotherapy, as well as patients with >3 positive ALNs on PET/CT and a tumour-negative MARI-node. An ALND is only performed in patients with >3 positive ALNs on PET/CT and a tumour-positive MARI-node.

Results In total 168 patients received a PET/CT and a MARI procedure, of whom 43% were hormone receptor positive, 28% triple negative and 29% Her2-positive. One hundred and eight patients (64%) showed ≤ 3 and 60 patients (36%) >3 suspected ALNs on PET/CT before the start of PST. The axillary pathologic complete response was 39%. In 134 patients (80%) an ALND was omitted; of these patients 94 (56%) were treated with axillary radiotherapy and 40 patients (24%) received no further axillary treatment. In 34 patients (20%) an ALND was performed (Table 1). The median number of positive additional nodes at ALND was 5 (range 0-16). During a median follow-up of 6 months there were no local recurrences.

Axillary treatment

Suspect ALNs on PET/CT	Outcome MARI	Axillary Treatment		
		None	Radiotherapy	ALND + Radiotherapy
≤ 3	Negative	40	-	-
	Positive	-	68	-
>3	Negative	-	26	-
	Positive	-	-	34
Total		40	94	34

ALN: axillary lymph nodes; ALND: axillary lymph node dissection; MARI: Marking the Axilla with Radioactive Iodine Seeds

Conclusion Combining pre-PST axillary staging with PET/CT and post-PST staging with use of the MARI-procedure results in a reduction of 80% of axillary lymph node dissections in breast cancer patients with clinical node-positive disease.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-08

Title: A nomogram to predict the survival benefit of surgical axillary staging in T1 breast cancer patients

Chen K, Zhu L, Li S, Su F and Song E. Breast Tumor Center, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China.

Body: Purpose:

Whether axillary staging (AS) surgery can be spared in selected breast cancer patients is unknown. The European Institute of Oncology of Milan initiated the SOUND trial[9] (Sentinel node vs. Observation after axillary UltraSOUND) and randomized T1 breast cancer patients with clinically negative axilla into SLNB vs. observation. Amy Cyret al. initiated a similar study (NCT01821768) and recently started to recruit patients in the United States. Consistent with the rationale of these two studies, we hypothesize that surgical axillary staging (AS), whether by SLNB and/or ALND, may not be associated with improved clinical outcomes in selected patients with a small tumor (T1). This retrospective cohort study developed a nomogram to predict the cancer-specific survival (CSS) of T1 breast cancer patients with and without AS and estimate the survival benefit of AS in these patients.

Method:

We used surveillance, epidemiology and end results (SEER) data to identify 232,195 breast cancer patients with T1 tumors diagnosed between 1990 and 2008. Patients with zero and 1 to 89 axillary lymph nodes examined were classified as the non-AS and AS groups, respectively. Patients who were diagnosed in even (1990,1992, etc) and odd (1991, 1993, etc) years were used as training and validation cohort, respectively. In the training cohort, we used the Kaplan-Meier method and competing risk analysis to screen for prognostic factors for CSS. A nomogram to predict the CSS, with receiving AS or not as one of the predictors, was developed and validated internally and externally, using the C-index and calibration plots. The survival benefit of AS for a specific patient can be estimated by the difference of two predicted CSS, when the patient were considered as having and not having AS.

Results:

With a median follow-up of 109 months, the CSS of the study population were 96.3%, 92.3% and 88.5% at 5, 10 and 15 years, respectively. In the training cohort, we used competing risk analysis, with non-CSS as the competing risk, to show that age, marital status, race, T-stage, N-stage, histology, grade, ER, PR, AS, breast surgery (breast conservation / mastectomy) and radiotherapy were significantly associated with CSS. A nomogram was developed based on these predictors and validated internally (C-index=0.707, 95% CI 0.702-0.712) and externally (C-index=0.704, 95% CI 0.698-0.710). The nomogram was well calibrated. With this nomogram, AS was predicted to have less than 2% benefit of 5yr-, 10yr- and 15yr-CSS in 60.6% (140599/232195), 15.5%(36074/232195) and 8.6%(20043/232195) of the entire study population, respectively.

Discussions and Conclusions:

The SOUND trial set the non-inferiority margin of 2.5% for 5-year DDFS when comparing the SLNB group with the observation group. In our study, the internally and externally validated nomogram predicted that 60.6% of the T1 patients may have less than 2% benefit of 5yr-CSS. We suggested that this nomogram may aid in individualized surgical decision-making by providing the predicted benefit of AS in T1 breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-09

Title: The development of nomograms to predict axillary lymph node status in breast cancer patients

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Body: Purpose: Prediction of the axillary lymph node (ALN) status preoperatively is critical in the management of breast cancer patients. Several predictive models (MSKCC, Paris and Shanghai model) of ALN status were developed to predict the probability of having ≥ 1 ALNs(+) (N1 or above). However, none of them can predict the probability of having ≥ 3 or ≥ 4 ALNs(+). This study aims to develop a new set of nomograms to accurately predict the probability of having ≥ 1 , ≥ 3 and ≥ 4 ALNs(+).

Methods: We searched the National Cancer Database to identify eligible female breast cancer patients with profiles containing critical information. Patients diagnosed in 2010-2011 and 2012-2013 were designated as the training and validation cohort, respectively. In the training cohort, we used logistic regression to investigate risk factors for positive ALNs. A new set of nomograms, based on these risk factors, was developed to predict the probability of having ≥ 1 , ≥ 3 and ≥ 4 ALNs(+). In the validation cohort, we used ROC analysis with bootstrap correction and calibration plots to assess the discriminative ability and accuracy of the nomograms, respectively, and compared them with previously reported nomograms.

Results: In the training cohort (n=102,454), we identified age, locations of lesions, ethnicity, T-stage, histology, presence of mixed histology, HER2, grade and LVI as significant risk factors for ≥ 1 ALNs(+), ≥ 3 ALNs(+) and ≥ 4 ALNs(+). ER and PR status were significant risk factors for ≥ 1 ALNs(+), but not for ≥ 3 ALNs(+) or ≥ 4 ALNs(+). Based on these risk factors, we developed a set of three nomograms that were predictive of the probability of ≥ 1 (Nomogram-A), ≥ 3 (Nomogram-B) and ≥ 4 (Nomogram-C) ALNs(+). In the validation cohort (n=104,199), the AUC values of the Nomogram-A, -B and -C were 0.802 (95% CI 0.799-0.805), 0.855 (95% CI 0.851-0.859) and 0.865 (95% CI 0.860-0.869), respectively. The average estimation errors between actual and predicted probabilities were 1.45%, 0.96% and 0.72% for Nomogram A, B and C, respectively. The AUC values of the MSKCC, Paris and Shanghai nomograms were 0.791 (95% CI 0.788-0.794), 0.781 (95% CI 0.778-0.784) and 0.769 (95% CI 0.766-0.773), respectively. The MSKCC, Paris and Shanghai nomograms exhibited a slightly worse calibration, with an average estimation error of 7.05%, 1.28% and 1.86%, respectively

Conclusion: We developed a set of nomograms to predict the probability of having ≥ 1 , ≥ 3 and ≥ 4 ALNs(+) in early stage breast cancer patients. Nomogram-A performed slightly better than MSKCC, Paris and Shanghai model, regarding the discriminative ability and prediction accuracy. Nomogram-B and C has been demonstrated to be discriminative and accurate. We believed this set of nomogram can be informative for clinical decision making for early stage breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-10

Title: Cumulative analysis of breast cancer sentinel lymph node identification rate by radioisotope: A 13-year experience

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Body: Purpose:

To investigate the breast cancer sentinel lymph node (SLN) identification rate by radioisotope navigation over the past 13 years at a single cancer centre institution.

Method:

Retrospective, breast cancer sentinel lymph node data in the past 13 years was collected. Between May 2002 to August 2008, sulphur colloid was the radioisotope agent used at the institution. Between August 2008 to August 2012, the radioisotope agent was switched to phytate. Then, between September 2012 to June 2015, injection method of phytate was switched from subdermal to intradermal. The SLN identification rate from each three periods were compared and analysed.

Results:

In a 13-year period (May 2002~June 2015), a total of 5455 breast cancer axillary sentinel lymph node samples were collected. Each surgeon's first 50 cases were excluded, thus a reduction to 5105 samples were derived. 155 cases were bilateral. Out of the 5105 samples, 40 did not undergo radioisotope mapping.

During the 13-year period, total successful SLN identification rate was 96.7%(4897/5065). During the sulphur colloid period (May 2002~August 2008), successful SLN identification rate was 99.1%(996/1005). During the subdermal phytate period (August 2008~August 2012), successful SLN identification rate was 95.1%(2200/2313). During the intradermal phytate period (September 2012~June 2015), successful SLN identification rate was 97.4%(1701/1747).

After injection of radioisotope agent, lymphatic scan mapping may fail. However, radioisotope signals may still be detected by radioisotope navigator intra-operatively. During the sulphur colloid period (May 2002~August 2008), mapping failure rate was 5.2%(52/1005). SLN identification rate by intra-operative navigator after scan mapping failure was 96.1%(50/52). During the subdermal phytate period (August 2008~August 2012), mapping failure rate was 21.7%(501/2313). SLN identification rate by intra-operative navigator after scan mapping failure was 79.0%(396/501). During the intradermal phytate period (September 2012~June 2015), mapping failure rate was 12.8%(223/1747). SLN identification rate by intra-operative navigator after scan mapping failure was 83.4%(186/223).

Conclusion:

Different radioisotope agent may affect the identification rate of axillary sentinel lymph nodes in breast cancer patients. Change of injection method, such as from subdermal to intradermal, may improve identification rate in use of same radioisotope agent. Even when lymphatics scan mapping failed, intra-operative radioisotope navigator may still successfully identify axillary sentinel lymph nodes and avoid unnecessary dissection due to mapping failure.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-11

Title: SentimagIC: A non-inferiority trial comparing super paramagnetic iron oxide vs. Tc99 and blue dye in the detection of axillary sentinel nodes in patients with early stage breast cancer

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Body: Background: Sentinel lymph node biopsy (SLNB), performed using radioisotope tracer with or without blue dye, is a highly accurate method for staging the axilla in early breast cancer. A radioisotope tracer with or without blue dye is the most commonly used technique for SLNB. Superparamagnetic iron oxide mapping agents detected by a handheld magnetic probe have been explored to overcome the disadvantages of the standard technique which include the short half-life, availability, handling and disposal issues for radioisotope, and the risk of allergic reactions to blue dye. Iron oxide mapping agents have been shown to be non-inferior to the standard technique in European studies. The SentimagIC trial was designed to establish the non-inferiority of a new formulation of the magnetic tracer, SiennaXP, to the combination of radioisotope and blue dye and was required to support a US regulatory submission.

Methods: Between January and December 2015, 160 patients with clinically node negative early stage breast cancer were recruited from six centers in the United States. Subjects received radioisotope injection then an intraoperative subareolar injection of SiennaXP and isosulfan blue dye prior to SLNB being performed. The sentinel node identification rate was compared between SiennaXP and the standard technique to evaluate concordance and non-inferiority.

Results: 147 procedures were completed in 147 subjects. A total of 369 histologically confirmed nodes were excised. The nodal detection rate was 94.3% (348/369) with SiennaXP and 93.5% (345/369) with the standard technique (difference 0.8%, 95% binomial confidence interval lower bound -2.1%). The per-subject detection rate was 99.3% (145/146) with SiennaXP and 98.6% (144/146) with the standard technique (one subject excluded due to not contributing any analyzable nodes). There were 22 subjects with positive SLNs, of whom 21 (95.4%) were detected by both SiennaXP and the standard tracers. In one subject, a positive node was not identified by any tracer, but was removed as clinically suspicious. The number of nodes excised per subject was 2.4 for both SiennaXP and for the standard combined technique.

Conclusion: This study showed SiennaXP is non-inferior to the standard dual technique of radioisotope and blue dye for axillary sentinel lymph node detection in early stage breast cancer and this provides a potential alternative to radioisotope and blue dye.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-12

Title: Development of prediction model for omission of sentinel lymph node biopsy in T1 breast cancer

Cho JN, Song EJ, Lee MH, Jung S-Y, Lee S, Kang H-S, Sim SH, Park IH, Lee KS, Kim YJ, Kim S-K, Kwon Y, Nam B-H and Lee ES. Center for Breast Cancer, Research Institute and Hospital, National Cancer Center, Goyang, Korea and Gyeongsang National University Hospital, Jinu, Korea.

Body: INTRODUCTION

Axillary sentinel lymph node (SLN) biopsy is a standard method for axillary nodal staging in the treatment of breast cancer. However, along with the trends to SLN performed only without additional axillary lymph node dissection, it's time to be considered omission of SLN for selective patients. We developed a prediction model to assess the negative probability of sentinel lymph node metastasis, specifically focus on the patients with clinical T1 breast cancer.

METHODS and MATERIALS

The study group consisted of 513 consecutive patients with clinical T1 breast cancer, who had undergone primary surgery between 2007 and 2012. The clinicopathologic factors and imaging modalities including breast ultrasound (US), magnetic resonance imaging (MRI), chest computed tomography (CT), and positron emission tomography (PET) were evaluated. Patients who fulfilled our inclusion criteria were randomized into experimental and validation set by 3:1 ratio. In the experimental group ($n = 256$), multivariate logistic regression analysis was used to analyze the association of each variable with the likelihood of SLN metastases. A prediction model was developed based on the patients in the experimental group and was validated with internal patient cohorts.

RESULTS

Of the 513 patients, 119 (23.1%) were found to have SLN metastases. In univariate analysis, presence of lymphovascular invasion ($P < 0.001$) and suspicious finding of preoperative image studies (US, PET, and MRI, $P < 0.001$) were independent positive predictors of SLN metastases. In multivariate analysis of experimental group, estrogen receptor status ($P = 0.012$), presence of lymphatic invasion ($P < 0.001$), and suspicious finding of preoperative image studies (US, PET, and MRI, $P < 0.001$) were each associated with involvement of SLN. A prediction model based on this analysis consists of 9 rows including 6 variables (age, estrogen receptor status, presence of lymphatic invasion, and results of preoperative US, PET or CT, MRI). The sum of assigned points for all six variables made corresponding value of negative probability of SLN metastasis. The accuracy of prediction model applied to the validation group, as measured by the area under the receiver operating curve was 0.789.

CONCLUSIONS

The prediction model developed here may be a useful tool to assess SLN involvement for clinical T1 breast cancer patients. And prospective study for additional validation of the prediction model is currently in preparation, exploring the possibility of SLN biopsy omission.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-13

Title: Can axillary lymph node clearance be avoided in women with node positive breast cancer receiving primary chemotherapy?

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Body: Background

For patients who receive primary chemotherapy for their early breast cancer the current practice for lymph node positive (LN) disease at presentation is in transition, with a drive towards sentinel LN biopsy (SLNB) rather than axillary node clearance (ANC) for patients who achieve a good response to primary chemotherapy. Boileau et al initially reported that approximately 30% of patients could potentially avoid clearance, but with a recommendation for further evaluation before including SLNB in guidelines for biopsy proven node-positive disease prior to primary chemotherapy for early breast cancer.

Methods

This was a retrospective single centre study. Examining the records of all patients who had received primary chemotherapy between January 2010 and October 2014.

Patients were identified through the Guy's Breast Cancer Database and chemotherapy prescribing system. To fully assess the LN status, all patients were cross-referenced with the electronic notes on our electronic noting system (MOSAIQ), radiology on Patient Archiving and Communication System (PACS) and histology on our Electronic Patient Record (EPR).

Results:

1526 patients were identified, of whom 156 underwent primary chemotherapy. 111 patients had suspicious nodes on imaging (ultrasound and/or MRI) and underwent LN biopsy. 69 patients had positive nodes pre-chemotherapy. 28 of these 69 patients (40.6%) had negative nodes at ANC, of these 14 (50%) had complete pathological complete response (pCR) in their primary tumour(s) of whom 12 (86%) had radiological CR prior to surgery. Of the 41 who remained positive only three achieved pCR of their primary tumour after chemotherapy.

22 patients were LN positive post primary chemotherapy, despite having been identified as initially LN negative. Of these 9 had a negative pre chemotherapy biopsy, and only 1 of these 22 patients had a pCR.

Discussion:

We have confirmed that ANC may be avoided in selected patients with LN involvement at presentation. In our series over 40% could have had SLNB instead. Factors supporting this approach include those patients who have an excellent radiological response to primary chemotherapy. Conversely, 22 of 87 (25%) had positive LN after chemotherapy having been initially thought to be LN negative at presentation, highlighting the possible need for multiple nodal sampling prior to chemotherapy as well as further nodal assessment after chemotherapy for complete staging.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-14

Title: Lymphatic microsurgical preventive healing approach (LYMPHA) for the primary prevention of lymphedema

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Body: Background/Objective

The incidence of breast cancer related lymphedema is as high as 40% in patients undergoing axillary lymph node dissection (ALND) and radiation. We report our experience performing lymphatic-venous anastomoses (LVA) using Lymphatic Microsurgical Preventive Healing Approach (LYMPHA) at the time of axillary node dissection performed in patients at highest risk for developing lymphedema. This preventative microsurgical procedure was first described by Boccardo, Campisi et al in 2009.

Methods

Female patients with node positive breast cancer requiring ALND were offered LYMPHA beginning January 2013. Exclusion criteria included allergy to lymphazurin blue dye, pregnancy and pre-existing lymphedema. Immediately following ALND a LVA was performed with optical magnification. Axillary reverse mapping (ARM) using blue dye injected in the ipsilateral upper arm allowed for the identification and preservation of afferent lymphatic vessels, 1-3 (mean 1.5) were sutured into a branch of the axillary vein distal to a competent valve. Limb volume was assessed via circumferential arm measurements and (L-Dex®) bio-impedance spectroscopy. Limb volumes and clinical exam were used to define lymphedema (LE).

Results

Over 42 months, 52 patients were consented for LYMPHA and 42 completed the LYMPHA procedure. The majority had locally advanced disease, 96% receiving chemotherapy (54% receiving neoadjuvant chemotherapy) and 63% receiving adjuvant radiation. In the 10 patients unable to undergo LYMPHA, all were early in each surgeon's experience. Among these patients, 6 had no suitable lymphatic identified, 3 had no suitable vein and one had extensive axillary disease precluding anastomosis. Of the 42 patients who successfully underwent LVA, 32 had modified radical mastectomy, and 10 patients had breast conserving therapy with ALND. Mean current follow-up is 22 months (range 2-42). 3 of the 42 LYMPHA patients (7.14%) developed clinically-apparent LE. Among the 10 patients without completed LYMPHA, persistent LE developed in 3 (30%). Two patients from each cohort had transient LE which resolved by their 6 month follow up visit. A retrospective review of our historic LE rate from 2009-2014 for patients undergoing ALND demonstrated a LE rate of 31.3%. All patients (32) who had post-operative lymphoscintigraphy demonstrated patency of their LVA. We estimate that performing LYMPHA added 30-45 minutes to operative time. No procedure-related complications were reported.

Conclusion

Data in our high-risk cohort of patients undergoing ALND shows that LYMPHA is feasible, safe, and practical method for the primary prevention of clinical lymphedema. This technique serves to significantly reduce the rate of clinical LE (7.1 vs 31.3 %) in breast cancer patients. Follow up is ongoing to evaluate the significance of transient lymphedema and bio-impedance measurement abnormalities in our patient population. As our experience grows, we anticipate that the majority of patients undergoing ALND would benefit from the LYMPHA procedure. Larger multi-institution and randomized trials are warranted to further evaluate the effectiveness of LYMPHA.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-15

Title: Clinical application of multimodal fluorescent-radioactive system in advanced breast cancer patients with neoadjuvant therapy; interim analysis for 65 patients

Lee S, Jung S-Y, Kim S-K, Lee ES, Lee MH, Park SJ, Kang H-S, Lee KS, Park IH, Sim SH, Kim Y, Kwon Y, Joo J and Kang SH. Center for Breast Cancer, National Cancer Center, Goyang, Republic of Korea; Biometric Research Branch, National Cancer Center, Goyang, Republic of Korea and Molecular Imaging & Therapy Branch, National Cancer Center, Goyang, Republic of Korea.

Body: Purpose

This study aimed to evaluate the identification rate and operation time of sentinel lymph node biopsy (SLNB) by a multimodal fluorescent-radioactive system (MFS) using a mixture of indocyanine green (ICG), radioisotope (RI) compared with the RI alone in advanced breast cancer patients with neoadjuvant therapy.

Methods

In this phase II randomized study, we enrolled 65 patients with advanced breast cancer with neoadjuvant therapy. Then, they received SLNB with either MFS or RI. We compared the identification rate, operation time of SLNB and number of sentinel lymph nodes. We also evaluated the safety. We analyzed the data of 65 patients for interim analysis.

Results

The mean age of the MFS group and RI group was 51.7 and 45.9 years ($p=0.024$), respectively. There were no differences in histopathological characteristics, including tumor size, node positivity, and hormone receptor status between two groups. The radiologic study showed the clinically complete response in axillary lymph nodes after neoadjuvant therapy. In MFS group, sentinel lymph nodes (SLNs) were identified in 30 patients and we could not identify the SLNs in one patient. In RI group, we identified the SLNs in 33 patients and also could not identify them in one patient. ($p=0.947$). The average numbers of SLNs in the MFS and RI group were similar. (2.32 ± 1.10 vs. 2.18 ± 1.57 , respectively; $p=0.668$) The operation time of SLNB was similar in the each group (11.65 ± 6.49 vs. 9.47 ± 6.26 , respectively, $p=0.174$) There were no complication, including allergic reactions, skin staining or necrosis.

Conclusions

This study is the randomized trial that compared MFS using ICG and RI and the conventional RI method for SLNB in the advanced breast cancer with neoadjuvant therapy. The multimodal fluorescent-radioactive system is a feasible and safe method for SLNB for the breast cancer patients with neoadjuvant therapy. We will evaluate the 130 patients for final analysis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-16

Title: Comparison of axillary nodal status between different defined clinically node negative breast cancer: Is ACOSOG Z0011 criteria applicable to preoperative cytologically negative axilla?

Liang Y, Chen X, Zhu Y, Wu J, Huang O, Zong Y, Zhu S, Gao W, He J, Zhu L, Chen W, Li Y and Shen K. Comprehensive Breast Health Center, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China and Rui Jin Hospital, Shanghai, China.

Body: Background Axillary ultrasound (AUS) and US-guided fine-needle aspiration (FNA) are now part of the preoperative axillary assessment. The ACOSOG Z0011 trial demonstrated axillary lymph node dissection (ALND) may be omitted in patients with T1-2 tumor, clinically negative axilla and 1-2 positive sentinel lymph nodes (SLN). But for clinically node negative patients with AUS suspicious but FNA negative axilla, whether they could receive the same surgical procedure and omit ALND referencing to Z0011 criteria as clinically node negative patients identified by AUS has not been illustrated. The purpose of this study was to evaluate potential differences in axillary lymph node (ALN) metastatic status between clinically node negative patients identified by AUS (AUS group) and FNA (FNA group). **Methods** Patients with T1-2 tumor and clinically negative axilla treated in Shanghai Ruijin Hospital from Jan 2013 to Dec 2015 were enrolled. ALN metastatic status were compared between AUS group and FNA group. **Results** A total of 1007 patients were included. Preoperative axillary status (AUS negative or FNA negative) did not differ significantly between ALN positive and ALN negative patients ($p = 0.170$). Similarly, ALN metastatic status (negative, 1-2 positive or ≥ 3 positive) did not differ significantly between AUS group and FNA group ($p = 0.405$)

table 1. ALN status of patients with different defined clinically node negative breast cancer

	AUS group N = 886 (%)	FNA group N = 121 (%)	P value
ALN status			0.405
Negative	740 (83.5)	95 (78.5)	
1-2 positive	125 (14.1)	125 (14.1)	
≥ 3 positive	21 (2.4)	4 (3.3)	

. Subset analysis of patients fulfilling Z0011 eligibility criteria showed no significant difference in nonsentinel lymph node (NSLN) metastasis rate ($p = 0.591$) and number of positive lymph nodes ($p = 0.777$) between AUS group and FNA group

table 2. Nodal status of 1-2 SLN positive patients with different defined clinically node negative breast cancer

	AUS group N = 138 (%)	FNA group N = 24 (%)	P value
NSLN			0.591
Positive	27 (19.6)	3 (12.5)	
Negative	111 (80.4)	21 (87.5)	
Number of positive lymph nodes			0.777
1	94 (68.1)	13 (9.4)	
2	31 (22.5)	4 (16.7)	
3 or more	13 (9.4)	2 (8.3)	

. **Conclusions** Patients with clinically node negative axilla identified by FNA were comparable to those identified by AUS in ALN metastatic status. Indicating that patients with preoperative FNA negative axilla could receive the same surgical procedure and

may omit ALND referencing to Z0011 criteria as preoperative AUS negative patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-17

Title: The combination of preoperative computed tomography lymphography and intraoperative fluorescence imaging navigation for sentinel lymph node biopsy of early breast cancer patients

Abe H, Teramoto A, Yamasaki K, Yoneda K, Ogawa M, Kawasaki M and Kameyama M. Breast Center, Bell Land General Hospital, Sakai, Osaka, Japan and Bell Land General Hospital, Sakai, Osaka, Japan.

Body: Background: Sentinel lymph nodes (SLN) biopsy has been established as a standard of care in the treatment of early breast cancer. This technique represents a minimally invasive, highly accurate method of axillary staging and is an alternative to conventional axillary lymph node dissection. The combination of the radioisotope and dye-staining methods is the most accurate way to identify SLN. We had reported feasibility and safety of a new technique of SLN identification using fluorescence imaging of indocyanine green (ICG) injection without any need for training. Recently, SLN identification using computed tomography lymphography (CTLG) has been reported in Japan. This study investigated a usefulness of the combination of CTLG and fluorescence imaging for SLN biopsy of early breast cancer patients.

Patients and method: Between January 2013 and March 2016, 296 breast cancer patients without clinical evidence of lymph node metastasis were treated. On the day before the operation, CTLG was performed using 64-row multidetector CT. We performed an intradermal injection in the periareolar area, using 4 ml of contrast agent with 1 ml of local anesthetic. The contrasted lymph route and SLN were identified in reconstructed three-dimensional imaging. The SLN spot was indicated by CT laser light navigator system. We established typical pattern of the lymphography: stain defect of SLN, stagnation of lymphatic route for preoperative diagnosis of metastatic SLN. During the operation, fluorescence images were obtained using the fluorescence imaging system, Photodynamic Eye (pde-neo, Hamamatsu Photonics Co., Japan). After 0.5 ml dye mixed indigocarmine and ICG was injected intradermally into the periareolar skin, lymphatic route was observed with fluorescence images. SLN biopsy was performed referring to the point by axillary compression technique by plastic device. Intraoperative pathological analysis of SLN was examined.

Results: The median age of the 296 patients was 59 (range 28 – 90) years old. One patient was male and others were female. CTLG and fluorescence imaging were safely performed in all patients. CTLG could visualize lymphatic route and accurately identify SLN in 284 (95.9 %) and 290 (98.0 %) cases, respectively, whereas fluorescence imaging identified successfully lymphatic route and SLN in all patients. Lymphatic routes of CTLG were completely consistent with those of fluorescence imaging. The number of SLN identified by CTLG was significantly lower than that by fluorescence imaging (1.1 vs. 1.6, $p < 0.01$). Thirty-nine of 296 patients had metastatic SLN pathologically, and 10 of them had micrometastases of SLNs. The accuracy for metastatic diagnosis of SLN using CTLG without micrometastasis was 83.9 %, sensitivity was 82.1 % and specificity was 84.1 %. The positive predictive value was 35.9 % and negative predictive value was 97.7 %.

Conclusion: The combination of CTLG and fluorescence imaging revealed easy and effective to detect SLN. The fluorescence imaging was more high detection rate and number of SLN than CTLG. Otherwise, preoperative diagnosis of SLN metastasis using CTLG would be useful to detect negative SLNs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-18

Title: Preoperative prediction of axillary lymph node status based on artificial neural network algorithm model

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Body: Background

Axillary lymph node status remains the single most significant prognostic factor for patients with primary breast cancer. Clinical and pathologic data have been used to develop statistical models to predict the axillary nodal status; the diverse accuracy may reflect the complexity of factors related to axillary metastasis.

Artificial neural network (ANN) is a computational method proposed as a supplement to standard statistical models for predicting complex biological phenomena. ANN is composed of artificial neurons and interrelated by synaptic weights, effective in multifactorial analysis and has the ability to explore underlying nonlinear relations of interconnected variables.

The aim of this study was to create an ANN-based preoperative decision tool for prediction of nodal axillary status (N0, N+ with 1-3 positive lymph nodes and N+ with ≥ 4 positive nodes). In the clinical setting, this may contribute to improved selection of patients for no axillary staging for those predicted with disease-free axilla (N0), sentinel node biopsy for patients with predicted 1-3 nodal metastases, and axillary lymph node dissection or neoadjuvant therapy for patients displaying four or more involved axillary lymph nodes.

Methods

The cohort constituted of consecutive patients diagnosed with primary breast malignancy between January 2009 and December 2012 at Skåne University Hospital in Lund, Sweden. The exclusion criteria were palpable axillary nodes or cytology-verified axillary metastasis and neoadjuvant chemotherapy. Data on mode of detection were retrieved. Clinical parameters included age, BMI, menopausal status and the location of the tumor within the breast. A breast pathologist extracted the histopathological variables of the tumor and lymph node. The ANN consisted of 3 layers, an input layer using 1-22 separate variables, one hidden layer, and a single node output layer, with the nodal status N0, macro-metastases N+1-3 and N+ ≥ 4 , respectively as output. The ANN was trained by back-propagation using gradient descent of a cross entropy error, for 2000 epochs. Area under the receiver operating characteristic curve (AUC) was used to assess the performance of the ANN-based predictive models for axillary nodal status. Evaluation was performed in a stratified 5-fold cross validation scheme, repeated 10 times. Sensitivity and specificity are given for a cutoff value corresponding to optimal balanced accuracy.

Results

The cohort consisted of 800 patients, classified into N0 64 % (n=514), N+1-3 positive nodes 29% (n=232) and N+ ≥ 4 positive nodes 7% (n=54). The AUC was 0.72 for prediction of node negativity; sensitivity 74% and specificity 64%. AUC was 0.77 for N+ ≥ 4 ; sensitivity 67% and specificity 79%. The predictive model for N+1-3 macro-metastases is in progress. Tumor size and lymphovascular invasion are the two principal risk variables selected to construct the ANN predictive model for N+ ≥ 4 . However, for N0, the predictive model is characterized by a complicated integration of numerous clinicopathological risk variables.

Conclusions

Based on clinicopathological and mammography-screening data, ANN can be valuable in predicting axillary nodal status and as a guidance tool for directing patients to personalized treatment of the axilla.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-19

Title: SentiNot: A way to avoid sentinel node biopsy (SNB) in patients with a preoperative diagnosis of ductal cancer in situ (DCIS)

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Body: Background

The risk for node metastasis in preoperative diagnosis of DCIS is low. ASCO guidelines suggest that SNB could be performed when mastectomy is planned or in those cases where the probability of upgrading to invasive cancer postoperatively is high. Despite that, SNB is performed almost in 54% of DCIS procedures in the Uppsala-Örebro Region. Thus, morbidity and resources have to be balanced against the risk of a reoperation.

Methods

Patients with a preoperative diagnosis of DCIS grade 3, grade 2 ≥ 20 mm or planned for a mastectomy will be included. Sienna+ is injected in the breast at the first operation. If the specimen contains invasive cancer, a SNB is performed in another session. Sienna+ can be detected in the axilla at least four weeks after the injection. Endpoints of the trial are the feasibility of detection of the SN as well as how many SNBs are avoided.

Results

In the first 34 cases, six had invasive breast cancer

Patient characteristics

	Total cohort (N=34)	Group with IBC (n=6)	
Age (yrs)	58.6 (54.9, 62.9)	60.2 (47.4, 72.9)	
Size (mm)	40.0 (31.2, 62.9)	57.7 (27.2, 88.1)	
DCIS grade			
	2	9 (26.5%)	2 (33.3%)
	3	23 (67.6%)	4 (66.7%)
	na	2 (5.9%)	0
Palpable			
	Yes	30 (88.2%)	1 (16.7%)
	No	4 (11.8%)	5 (83.3%)
BCS			
	Yes	22 (64.7%)	3 (50%)
	No	12 (35.3%)	3 (50%)
Transcutaneous counts at the end of the operation	266 (196,336)	300 (126,474)	

. On reoperation (median 29 days, iqr 10) SNB was successful in four cases with SPIO, one with Tc⁹⁹ and three cases with blue dye. Totally, the combination of SPIO and blue dye was successful in all cases. No metastases were found.

Conclusion

It is possible to load the SN with SPIO and reoperate within 4 weeks from the first operation. A rough 83,4% of all patients with DCIS who would have undergone SNB avoided it. Our preliminary results show that 5,6 SNB was avoided for each patient that

was operated. SN was detected in all cases with a combination of SPIO and blue dye, but only in 66,7% SPIO. It seems that increased experience with SPIO will increase the effectivity of this procedure.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-20

Title: The non-invasive treatment for sentinel lymph node metastasis by photodynamic therapy using a verteporfin solubilized phospholipid polymer aggregate

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Body: Introduction:

Sentinel lymph node biopsy (SLNB) has become a standard procedure for axillary lymph node evaluation in clinically node-negative breast cancer patients. Recent trial suggested that patients with 1 or 2 sentinel lymph nodes (SLNs) involvement could be treated with SLNB alone.

Although SLNB is much less invasive procedure comparing with axillary lymph node dissection (ALND), it is still associated with complications such as lymph edema, numbness and pain.

Photodynamic therapy (PDT) against cancer is a non-invasive optical therapeutic method in which the topical or systemic delivery of photosensitizing drugs is followed by its subsequent activation with broadband red light.

In this study, the usefulness of PDT for treating SLN metastasis was evaluated in murine model.

Materials and Methods:

Verteporfin, a hydrophobic photosensitizer (PS) forms a soluble conjugate in aqueous medium with a water-soluble and amphiphilic PMB polymer as a solubilizer. The PMB forms stable and well-dispersed molecular aggregate when its concentration is over 1.0 mg/mL based on the hydrophobic interactions among polymer chains. The verteporfin can form conjugate (PMB-verteporfin) with hydrophobic domain in the PMB aggregate. The PMB-verteporfin was injected at dorsum manus of BALB/c nude mice. The concentrations of verteporfin in tissues were determined by measuring the fluorescence emitted at 700 nm (with excitation at 430 nm). To develop a murine SLN metastasis model, 5×10^5 human epidermoid carcinoma A431 cells with stable expression of GFP were injected to the forearm of BALB/c nude mice. Seven days after inoculation of cancer cells, 20 μ L of PMB-verteporfin was injected at dorsum manus of BALB/c nude mice and 75 J of light energy was delivered using a 640 nm diode laser for a total treatment time of 1 min. Fifty-three mice were randomly assigned to the combination of PMB-verteporfin injection and light exposure (A), light exposure alone (B), PMB-verteporfin injection alone (C), and no treatment (D) groups. Ten days after PDT, brachial lymph nodes, which were considered as SLNs were harvested and evaluated by stereoscopic fluorescence microscope. And, DNA was extracted from harvested lymph node. Human Alu family sequence was detected by 7300 Real Time PCR system (Applied Biosystems, Carlsbad CA USA) to estimate metastatic volume.

Results:

The concentration of verteporfin in SLN was significantly higher than other organs including lung, liver, kidney and brachial skin. The group A significantly reduced the SLN metastasis (13%) comparing with , group B (57%), group C (46%) and group D (52%). The Ct value in a PCR of the combination of group A (Ct=29.17) significantly reduced the SLN metastasis comparing with group B (Ct=22.45, p=0.018), group C (Ct=25.58, p=0.018) and group D (Ct=25.54, p=0.005).

Conclusions:

These data suggested that PDT using PMB as a nanotransporter of verteporfin could be a minimally invasive treatment of SLN metastasis in breast cancer, and represent a potential alternative procedure to SLNB.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-21

Title: Preoperative axillary ultrasound guided needle sampling in breast cancer: Comparing the sensitivity of fine needle aspiration cytology and core needle biopsy

Topps AR R, Barr SP P, Pritchard S and Maxwell AJ J. Nightingale Centre and Genesis Prevention Centre, University Hospital of South Manchester, Manchester, United Kingdom and Centre for Imaging Studies, University of Manchester, Manchester, United Kingdom.

Body: Background: Preoperative axillary ultrasound (US) combined with selective US-guided needle sampling (UNS) can be used to identify lymph node metastases. This can inform decisions about neoadjuvant chemotherapy and allow a patient to proceed immediately to axillary lymph node dissection (ALND) thus avoiding an extra sentinel node biopsy (SNB) procedure. We acknowledge the landmark ACOSOG Z0011 trial showing a subgroup of patients (T1-2) undergoing breast conserving surgery and whole-breast radiotherapy in which ALND can safely be omitted if they have minimal nodal disease burden. For these patients the utility of UNS may be limited if the surgeon has modified their practice according to the trial. For patients not fitting the Z0011 trial criteria, preoperative UNS remains important.

Previous studies comparing the sensitivity of axillary US-guided fine needle aspiration cytology (FNA) and core needle biopsy (CNB) have been small and a meta-analysis has not shown a difference in sensitivity¹. Our aim was to directly compare the sensitivity of the two techniques.

Method: Patients with macrometastatic nodal involvement that were treated at a tertiary referral centre between January 2013 and December 2014 were retrospectively identified from pathology records. Preoperative UNS had been performed by one of eight Consultant Radiologists with the sampling method being according to each individual radiologist's preference. The result of the first UNS performed on each patient was compared to post-operative histopathology results. Patients who had undergone previous axillary surgery or any part of their investigations/treatment at another unit were excluded.

Results: A total of 101 CNBs and 181 FNAs were performed in 282 patients. There were 78 true positive CNBs and 96 true positive FNAs. US-guided CNB was therefore more sensitive than US-guided FNA (77.2% vs. 53.0%, $p < 0.001$). Two non-diagnostic CNBs and eight non-diagnostic FNAs were performed. Five patients in the CNB group were correctly identified preoperatively as having isolated tumour cells (ITCs) or micrometastatic disease only in their axillary lymph nodes and were therefore triaged to SNB rather than ALND. A single haematoma requiring non-operative management was recorded in the CNB group.

Conclusion: US-guided CNB of the axilla is more sensitive than US-guided FNA and is a safe technique in experienced hands. We also highlight the additional potential benefit (whilst accepting the possibility of sampling error) of CNB over FNA in assisting the multidisciplinary planning of axillary surgery in patients who are found to have ITCs or micrometastatic disease only during their preoperative axillary staging.

References: Houssami N, Ciatto S, Turner RM, *et al.* Preoperative ultrasound-guided needle biopsy of axillary nodes in invasive breast cancer: meta-analysis of its accuracy and utility in staging the axilla. *Ann Surg* 2011;254:243-251.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-22

Title: The long term outcomes of Metasin RTqPCR intra-operative sentinel node analysis in early breast cancer

Udayasankar S, Ashley E, Jenkins KS S, Huws A, Sharaiha Y, Sai-Giridhar P, Thomas D, Munir A, Holt S and Khawaja S. Prince Phillip Hospital, Llanelli, Wales, United Kingdom.

Body: Background:

Axillary lymph node involvement is a prognostic factor in breast cancer and it is used to guide adjuvant therapy. Axillary clearance remains the standard of care in lymph node positive disease in most parts of the world. Usually this is performed as second procedure but immediate intra-operative node analysis allows clearance to be performed as part of the initial procedure where necessary.

The Metasin assay targets the breast epithelial cell markers CK19 and mammaglobin mRNA and detects the presence of breast tissue (metastatic disease) in the sentinel nodes. Evidence shows the Metasin assay to be fast (average assay time 41.2min) and accurate with a discordance rate below 4% compared with histology. The cost effectiveness of the assay has been reported in our previous studies.

Aim:

The aim of this study is to assess the risk of axillary recurrence following the use of the Metasin assay to guide axillary management.

Method:

This is a single centre retrospective study which included all patients presenting to a district general hospital with early clinically node negative breast cancer undergoing sentinel node biopsy between Oct 2011 and Dec 2014.

Alternate 2 mm slices of the node were examined intraoperatively using the Metasin assay and the remainder sent for histological examination. The results of the Metasin assay and histology were compared. The risk of axillary recurrence using the Metasin test to select patients for immediate axillary clearance was assessed.

Results:

1073 sentinel nodes from 545 patients were analysed during this three-year period. 2 patients were lost to follow up. 94 patients were node positive and underwent axillary clearance as part of their primary surgery. 449 patients had sentinel node biopsy with no further axillary procedure. There were 36 nodes (3.34%) with discordant results. Median follow up was 32 months (range 18 to 55 months).

Fourteen patients presented with recurrences (2.56%). The mean event free interval was 15 months. Of the 14 patients, 5 patients tested with Metasin had macrometastases and underwent immediate axillary clearance during the primary surgery. 7 patients were node negative and 2 patients were shown to have micrometastases. 11 patients recurred with distant metastases, 3 patients with local recurrence and 2 patients (0.36%) with axillary recurrences.

The two axillary recurrences occurred at 3 and 5 months after primary surgery. Both patients underwent Metasin intraoperative analyses of sentinel nodes which were negative. Subsequent histological examination confirmed no metastatic node involvement in one patient but micro-metastases in the other. Both of these patients had aggressive disease with local, axillary and distant metastasis and subsequently died of their disease.

Conclusions:

The Metasin assay is a reliable intraoperative test for sentinel node involvement and when used to guide surgical axillary management is associated with very low axillary recurrences (0.36% with a median follow up of 32 months).

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-23

Title: Long-term follow-up of persistent breast dermopigmentation after sentinel lymph node identification using superparamagnetic iron oxide particles (SIENNA+®)

Hannebicque K, Boulanger L, Bogart E, Giard S, Chauvet MP and Houpeau JL. Centre Oscar Lambret, Lille, France.

Body: Background

The French Sentimag study evaluated a non-invasive method for the localization of breast cancer sentinel lymph nodes (SLN) using SIENNA+®, a superparamagnetic iron oxide particles (SPIO), in addition to conventional techniques (radiotracer and blue dye). SIENNA+® was injected subcutaneously into the breast and detected by the SENTIMAG® handheld magnetometer probe. The results showed a good SLN identification performance but a skin discoloration was noted during this study after the SIENNA+® injection. This aim of this study was to assess the long-term duration and appearance of this dermopigmentation.

Methods:

56 patients had participated in Sentimag study in our Center, 6 patients who had undergone mastectomy were excluded. We selected 50 patients who had undergone breast conservative surgery. For these patients, SLN localization was performed by both the conventional method (radiotracer and /or blue dye) and magnetic tracer, SIENNA+®. 47 patients were reviewed retrospectively from January 2015 to April 2015, 1.5 to 2 years after surgery and were assessed for skin discoloration.

Results :

Of the 47 patients, a dermopigmentation, from grade 1 (light yellowing) to grade 3 (dark brownning) remained visible at the site of injection of SIENNA+® after 20.2 months [14.4-25.9] in 36.1% of the patients (17/47). 6.4% of 47 patients seen had grade 3 skin discoloration and 29.7% had grade 1 or 2 skin discoloration. Interestingly, no patients reported that persistent staining was a cosmetic or psychological problem.

Conclusions :

The use of SIENNA+® appears as an alternative method to radioisotopes for SLN identification in early breast cancer, but it may result in a prolonged-dermopigmentation at the injection site. To avoid dermopigmentation, it would be interesting to compare different techniques of SIENNA+® injection into the breast (intra-tumoral injection or a deeper periareolar injection) through a randomized trial.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-24

Title: Evaluating lymph node ratio (LNR) as a prognostic indicator in node-positive breast cancer

Kelliher A, Boyce M, Donnellan E, O Connor D, Murphy C and Bird B. University College Cork, Cork, Ireland and Bon Secours Hospital Cork, Cork, Ireland.

Body: Purpose: To determine whether LNR can provide additional prognostic information to pN staging in node-positive breast cancer patients.

Methods: We previously reported on the utility of LNR as a prognostic indicator in lymph node-positive early breast cancer. We present an updated analysis with a prolonged median follow-up of 85 months. A retrospective review of original histopathology reports was undertaken in a sample group of 214 patients diagnosed with node-positive breast cancer between January 2000 and January 2011. The LNR was defined as the number of positive lymph nodes divided by the total number of lymph nodes (LNs) removed. LNR cut off points were divided into low (≤ 0.14), intermediate (0.15-0.39) and high (≥ 0.40) by trichotomy. Patients were also divided into three groups based on nodal status in accordance with the AJCC staging system. Those with 1-3 positive nodes were classified as pN1, those with 4-9 positive nodes were pN2 and those with 10 or more positive nodes were classified as pN3. Overall survival (OS) and invasive Disease Free Survival (iDFS) were calculated using the Kaplan Meier Method.

Results: The mean age at diagnosis was 58 (SD=12) years. The median follow-up time was 85 months. The 5-year OS and iDFS for node-positive patients were 80% and 70%, respectively. The mean number of lymph nodes examined was 15.2 (SD=6.6) and the mean number of positive nodes was 5.3 (SD=6.1).

When patients were stratified according to LNR groupings OS was 80% in the low LNR group, decreasing to 67% in the intermediate LNR group and 47% in the high LNR group ($p < 0.0001$). Similarly, iDFS in the low LNR group was 71% decreasing to 57% in the intermediate LNR group and 42% in the high LNR group ($p < 0.0009$).

Conclusions: These updated results from a cohort of patients with lymph node-positive early breast cancer at a median follow-up of over 7 years confirms the utility of LNR as a prognostic indicator. Univariate and multivariate analyses to assess the ability of LNR to discriminate risk in addition to AJCC nodal grouping will be presented.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-25

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-26

Title: Validation of a Chinese nomogram with a Dutch breast cancer population: Excellent prediction of the probability of axillary lymph node metastasis

Qiu S-Q, Aarnink M, van Maaren MC C, Dorrius M, Koffijberg H, van Dam GM M and Siesling S. University of Groningen, University Medical Center Groningen, Groningen, Netherlands; The Breast Center, Cancer Hospital of Shantou University Medical College, Shantou, Guangdong, China; MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, Overijssel, Netherlands; Netherlands Comprehensive Cancer Organisation, Utrecht, Netherlands; University of Groningen, University Medical Center Groningen, Groningen, Netherlands and University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

Body: Background: In the era of precision medicine, the surgical management of axillary lymph nodes (ALN) should be patient-tailored. Omission of sentinel lymph node biopsy (SLNB) is possible in patients with early breast cancer and very low or very high probability of ALN metastasis. Recently, we developed a nomogram to predict the probability of ALN metastasis in breast cancer patients based on clinicopathological parameters including ultrasound using a Chinese patient dataset¹. In this study the nomogram performance was validated in an independent Dutch population from one hospital.

Methods: Data of 170 Dutch patients with a successful SLNB or axillary lymph node dissection were collected. A lymph node containing either micro- or macrometastatic disease was considered as a positive lymph node. Performance of the nomogram was assessed by calculating the area under the receiver-operator characteristic (ROC) curve (AUC). False-negative rates (FNRs) and false-positive rates (FPRs) at several different predictive cut-off points were calculated.

Results: There were 69 (40.6%) patients having a positive ALN. The AUC for the nomogram was 0.84 (95% confidence interval 0.78-0.90) compared with 0.86 in the Chinese validation population, showing excellent discrimination of the model. The FNR and FPR of the model were 10.2% and 0% for the predicted probability cut-off points of 14.5% and 90%, respectively.

Table 1 False-negative rates (FNRs) and false-positive rates (FPRs) of the nomogram at different predictive cut-off points

Predicted risk	Patient number and percentage (%)	Number of patients with positive ALN	FNR (%)
< 14.5%	59 (34.7)	6	10.2
< 20%	79 (46.5)	11	13.9
		Number of patients with negative ALN	FPR (%)
> 70%	27 (15.9)	1	3.7
> 90%	18 (10.6)	0	0

ALN: axillary lymph node

This means that omission of SLNB is possible for patients with a predictive probability of less than 14.5% or higher than 90%, which accounts for 45.3% of all patients in this study.

Conclusions and future perspectives: In this study, the Chinese nomogram showed excellent performance in predicting the probability of ALN metastasis in an independent Dutch population. A multicentre validation of this nomogram in large Dutch patient population (>2500 patients) is ongoing.

Reference

1. Qiu S-Q, Zeng H-C, Zhang F, et al. A nomogram to predict the probability of axillary lymph node metastasis in early breast cancer patients with positive axillary ultrasound. *Sci Rep* 2016; 6: 21196.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-27

Title: Sentinel lymph node biopsy by contrast-enhanced ultrasonography with sonazoid in patients with breast cancer - Prospective multicenter study

Shimazu K, Ito T, Uji K, Miyake T, Motomura K and Noguchi S. Osaka University Graduate School of Medicine, Suita, Osaka, Japan; Rinku General Medical Center, Izumisano, Osaka, Japan and Osaka General Medical Center, Osaka, Japan.

Body: Background: The aim of this prospective study is to evaluate feasibility of the periareolar injection of contrast agent (Sonazoid (SNZ)) followed by ultrasonography (US) for identification of sentinel lymph node (SLN) in breast cancer patients with clinically negative node.

Patients and Methods: Patients with T1-2N0M0 breast cancer were recruited in this study. They received the periareolar injection of SNZ followed by US to identify contrast-enhanced SLN. Fine needle aspiration biopsy (FNAB) was done for each CE-SLN. Then, they underwent SLN biopsy with the conventional method, blue dye and/or radiotracer (B/R).

Results: In almost all cases, contrast-enhanced lymphatic vessels were clearly visualized US soon after the periareolar injection of SNZ, and SLN, into which lymphatic flow was draining, was easily identified. The identification rate of SLN was 98% (98/100) by SNZ and 100% (100/100) by B/R. The number of SLNs identified by SNZ (mean per patient, 1.52) was significantly ($P < 0.001$) lower than that of those by B/R (2.22). Twenty-five patients had at least one metastasis in the SLNs identified by SNZ and/or B/R. In these patients, SLNs ($n=39$) identified by both SNZ and B/R showed a significantly ($P < 0.0001$) higher positivity (74.4%) for metastases than those ($n=19$) identified by B/R alone (21.1%).

Conclusion: Identification of SLN by periareolar injection of SNZ followed by US is a technically easy method and the identification rate of SLN was as high as 98%, being comparable to the conventional B/R. SLNs detected by SNZ seem to represent the true SLNs which first receive lymphatic flow from the tumor among the SLNs detected by B/R.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-28

Title: Intraoperative assessment of the sentinel node (SN) in breast cancer by one step nucleic acid assay (OSNA): Experience of over 800 patients

Clarke D, Weston S, Ion C, Smith A, Kabir A, Harries S and Jones L. Warwick Breast Unit, South Warwickshire NHS Foundation Trust, Warwick, Warwickshire, United Kingdom.

Body: Introduction

The intra-operative assessment of the sentinel node in women with breast cancer enables an immediate axillary node clearance if the sentinel node is positive. This has significant benefits for the Patient, the Surgeon and the Health Care Provider. There are a variety of methods for the intra-operative assessment which include: Touch Imprint Cytology, Frozen Section Analysis and Polymerase Chain Reaction (PCR) based molecular assays. OSNA is a molecular assay using PCR, which detects the presence of cytokeratin 19 in the sentinel node. We report our experience of OSNA for the intraoperative assessment of the sentinel node in a single institution with 807 patients.

Methods

All patients with operable breast cancer who were found to be node negative on clinical and radiological assessment of the axilla, and who had their axilla staged by a sentinel node biopsy at the Breast Unit at Warwick Hospital, UK over a 5 year period were included in this study. Data was collected from a prospective database maintained at the Breast Unit. The axillary node positivity rate and the percentage of patients with macrometastatic and micrometastatic disease as detected by OSNA was collected and compared with a group of 411 patients who had the intraoperative assessment by Touch Imprint Cytology and final histology by conventional Haematoxylin & Eosin (H&E) assessment, prior to the introduction of OSNA. The Chi-square test were used for statistical significance.

Results

807 patients had their sentinel assessed intraoperatively using OSNA in this 5 year study period. The sentinel node was positive in 292 patients (36.5%). Of those who had a positive node, 138(17.3%) had macrometastatic disease and 154(19.2%) had micrometastatic disease. When compared to 411 patients in the preOSNA period, that were assessed by Touch Imprint Cytology and H&E sections, the node positivity rate increased from 24.6% to 36.5% (p 0.0001) with the introduction of OSNA. Whilst there was no significant increase in the rate of macrometastatic disease – 21.15% versus 17.3 % (p 0.052), there was a significant increase in the patients who had micrometastases detected on OSNA - 3.5% versus 19.2 % (p 0.0009) as shown in the table.

Comparison of OSNA with conventional Touch Imprint Cytology and H&E

Result		TIC and H&E	TIC and H&E	OSNA	OSNA	p value
		Number	Percentage	Number	Percentage	
Positive	Macro	84	21.1	138	17.3	0.052
Positive	Micro	14	3.5	154	19.2	0.0009
Negative		313	65.4	515	63.5	

Conclusion

Our results prove that OSNA is a much more sensitive test for picking up metastatic disease, especially micrometastatic disease, in the sentinel node. Whilst this did cause some anxiety in the initial part of the study period, the results of recent trials like ACSOG Z-11 and IBCSG 23-01 have shown that small volume disease or micrometastases in the sentinel node do not always require an axillary node clearance. Intraoperative assessment of the sentinel node with OSNA significantly upstages the axillary nodal status, especially with regard to micrometastatic disease, but the ability to proceed to an axillary node clearance at the same operation as the sentinel node biopsy, still has significant advantages for the Patient, Surgeon and Health Care Providers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-29

Title: In the era of conservative surgery, can patients presenting with node positive breast cancer be spared axillary node dissection post neoadjuvant chemotherapy? A meta-analysis and review of literature

Headon H, Chehade HEH, El Tokhy O, Wazir U, Heeney J, Kasem A and Mokbel K. London Breast Institute, London, United Kingdom.

Body: Background: The use of sentinel lymph node biopsy (SLNB) following neoadjuvant chemotherapy (NAC) in patients presenting with clinically positive lymph nodes remains controversial.

Methods: A computer-aided search of the literature regarding SLNB in clinically node-positive breast cancer treated with NAC was carried out to identify the false negative rate (FNR), sentinel lymph node identification rate (IR), and axillary pathological complete response (pCR).

Results: Nineteen articles were used in the analysis yielding 3398 patients. The pooled estimate of the FNR was 13% and that of the IR was 91%. The adjusted pCR rate was 47%.

Conclusions: SLNB after NAC in biopsy-proven node positive patients results in reasonably acceptable FNR and IR making it a valid alternative management strategy to axillary dissection. Although the results are not matched with those in clinically node negative patients, a FNR of 13% is very unlikely to adversely affect overall survival. Its impact on locoregional recurrence should be evaluated in adequately powered future studies. More refined patient selection and optimal techniques can improve the FNR and IR in this patient population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-30

Title: Limited effectiveness of patent blue dye in addition to isotope scanning for identification of sentinel lymph nodes: Cross-sectional real-life study in 1024 breast cancer patients

Merlin J-L, Rauch P, Leufflen L, Salleron J, Harlé A, Olivier P and Marchal F. Institut de Cancérologie de Lorraine, Nancy, France; Université de Lorraine, Nancy, France; CNRS UMR7039 CRAN, Vandoeuvre les Nancy, France and CHU, Nancy, France.

Body: Background. Reduced morbidity is associated with sentinel lymph node (SLN) biopsy performed with isotopic and blue dye identification. However, because its use has been associated with side effects including anaphylactic reactions, the effectiveness of adding blue dye to radioisotope for SLN mapping remains debated.

Patients and Methods. Using data from a prospectively maintained database, 1884 lymph node-negative breast cancer patients who underwent partial mastectomy with SLN mapping by a dual-tracer using patent blue dye and radioisotope, using the same subareolar injection technique, were prospectively studied in a real-life setting between January 2000 and July 2013. A total of 1024 with tumors <3 cm and with >1 node detected by one of the two techniques (N=1024) were included in this cross-sectional study. A lymph node was considered as an SLN when it was stained with patent blue (either partial or complete), had a blue lymphatic afferent, or had increased radioactivity. SLNs identified during these procedures were classified as containing both blue dye and radiotracer ("blue-hot" nodes), radiotracer alone ("hot-only" nodes), or blue dye alone ("blue-only" nodes).

Results. Among the 1024 patients analyzed for SLN mapping, 1010 patients (98.6%) had at least one hot SLN, and 880 (86.0%) had at least one blue SLN. A total of 866 patients (84.6%) had at least one blue-hot SLN. Only four patients had blue-only nodes involved and 26 patients had hot-only nodes. Therefore, the failure rate of the isotopic and the colorimetric method were 1.5% (4/274) and 9.5% (26/274), respectively. Among these four patients having no detectable radioactivity in the axilla, two had negative lymphoscintigraphy. Therefore, the contribution of blue dye to metastatic nodes was relevant for the identification of only 2/274 patients (0.8%). In the univariate analysis, age<60 ($P=0.0089$), tumor size ($P<0.0001$), tumor grade ($P=0.0436$), and blue SLNs ($P=0.0109$) were significantly associated with node involvement. In the multivariate analysis, after adjusting for the number of identified SLNs, blue SLNs remained significant ($P=0.0210$) but the discriminant power of this multivariate model was low (AUC=0.66). Three patients (0.3%) had an allergic reaction with patent blue dye, and anaphylactic shock occurred in two cases (0.2%).

Conclusions.

The patent blue dye method is less sensitive than isotopic method to assess SLN involvement. Its added-value to reduce the false-negative rate is only limited to the rare cases in which no radioactivity is detectable in the axilla (<1%). When a radioisotope mapping agent is available, the use of patent blue dye should be avoided, because it can induce anaphylaxis. The results of this real-life cross-sectional study performed in a large number of patients, bring additional data that confirm the findings of previous studies. Highlighting the low interest of adding patent blue dye to isotopic detection for SLN mapping, these results led us to modify our procedures: when a radioisotope mapping agent is available, the use of blue dye is now avoided, because it can induce allergic reactions that can be life-threatening.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-31

Title: Validation of novel diagnostic kits using the semi-dry dot-blot method for detecting metastatic lymph nodes in breast cancer; distinguishing macrometastases and micrometastases

Otsubo R, Hirakawa H, Oikawa M, Inamasu E, Baba M, Matsumoto M, Yano H, Kinoshita N, Abe K, Fukuoka J and Nagayasu T. Nagasaki University Hospital, Nagasaki, Japan; Chiba Aiyukai Memorial Hospital, Chiba, Japan and Nyuuwakai Oikawa Hospital, Fukuoka, Japan.

Body: Background: The semi-dry dot-blot (SDB) method is a diagnostic procedure for detecting lymph node (LN) metastases. Metastases are confirmed by the presence of cytokeratin (CK) in lavage fluid of sectioned LNs that contain anti-pancytokeratin antibody, based on the theory that epithelial components such as CK are not found in normal LNs. We evaluated two novel SDB kits that use the newly developed anti-CK19 antibody for diagnosing LN metastases in breast cancer.

Methods: We obtained 159 LNs dissected from 93 breast cancer patients from July 2013 to December 2015 at Nagasaki University Hospital, including 38 dissected axillary LNs and 121 sentinel LNs, sliced at 2-mm intervals and washed with phosphate-buffered saline. The suspended cells in the lavage fluid of sliced LNs were centrifuged and lysed to extract protein. This extracted protein was used with a low-power and a high-power kit to diagnose LN metastasis. The washed LNs were blindly diagnosed by pathologists using hematoxylin and eosin (H&E) stain. Diagnoses based on the kit were compared with their H&E counterparts.

Results: Of the 159 LNs, 68 were assessed as positive and 91 as negative by permanent pathological examination with H&E. Sensitivity, specificity, and accuracy of the low-power kit for detecting LN metastases was 83.8%, 100%, and 93.1%, respectively. In 11 false-negative cases, there were nine micrometastases, producing a sensitivity of 96.4% for detecting macrometastases. Sensitivity, specificity, and accuracy of the high-power kit for detecting LN metastases was 92.6%, 92.3%, and 92.5%, respectively. Combining the low- and high-power kit results, sensitivity, specificity and accuracy for distinguishing macrometastases from micrometastases was 94.5%, 95.2%, and 95.0%, respectively. Diagnosis was achieved in approximately 20 min using the kits, at a cost of less than 25 USD.

Conclusions: The kits in our study were accurate, quick, and cost-effective in diagnosing LN metastases without the loss of LN tissue. The kits' ability to distinguish macrometastases from micrometastases was excellent, which is important, clinically.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-32

Title: Second sentinel lymph node biopsy for patients with local recurrence after breast cancer surgery

Park JY, Song JH, Choi JE and Lee SJ. Yeungnam University Medical Center, Daegu, Republic of Korea.

Body: Background: Sentinel lymph node biopsy(SLNB) has become standard procedure for primary breast cancer patients who have no tumor metastasis in sentinel lymph node(SLN). In this study, we evaluated feasibility and pathologic outcomes of second SLNB in patients with locally recurrent breast cancer and their follow-up results.

Methods: From January 2008 to December 2015, 114 patients underwent operation for locally recurrent breast cancer. In 42 patients of them, lymphatic mapping was performed for second SLNB. When SLN was visualized in lymphangiography, SLNB was performed. In the cases where SLN metastasis was confirmed, axillary lymph node dissection(ALND) was performed. Follow-up studies were performed every 6 months for 5 years and then annually.

Results: The mean interval to local recurrence from the initial surgery of breast cancer was 64.6 ± 53.1 months. In 38 of 42 patients(90.5%), lymphatic mapping was successfully performed. There was no significant difference of success rate of lymphatic mapping according to previous operation method of breast and axilla or history of radiation therapy.

Aberrant lymphatic pathway was observed in 15 of 38 patients(39.5%). The rate of aberrant lymphatic pathway was higher in patients who underwent ALND previously than in patients who underwent SLNB only(81.8% vs 22.2%, $p=0.001$). In 6 patients who previously underwent ALND followed by radiation therapy, all their lymphatic pathway was altered. There was no significant difference of the rate of aberrant lymphatic pathway according to previous operation method of breast.

Of 38 patients in whom lymphatic mapping was successfully performed, 37 patients underwent SLNB. SLNs were identified in 31 patients(83.8%). There was no significant difference of success rate of SLNB according to previous operation method of breast and axilla or history of radiation therapy.

Of 31 patients whose SLNs were identified, 4 patients(12.9%) had tumor metastasis in their SLN. Among them, 3 patients underwent ALND but SLN was the only lymph node(LN) in which tumor metastasis was confirmed. The other patient underwent no further ALND because ipsilateral internal mammary LNs were only LNs in which tumor metastasis was confirmed in frozen section biopsy and micrometastasis was additionally confirmed in only one contralateral axillary LN in permanent biopsy. The mean follow-up period after operation for local recurrence was 33.0 ± 24.5 months. There were 10 cases(23.8%) of loco-regional recurrence or distant metastasis at 14 months of mean follow-up. Among them, one patient had ipsilateral axillary recurrence solitary at 11 months of follow-up. The patient underwent breast conserving surgery and SLNB for primary breast cancer which stage was I. Second SLNB for local recurrence was tried at 48 months after first operation but SLN was not identified. Because there was no evidence of axillary LN metastasis in preoperative image study, no further ALND was performed.

Conclusion: Second SLNB should be considered for patients with locally recurrent breast cancer because occult LN metastasis could be identified in the ipsilateral axilla or other site through aberrant lymphatic pathway. Further studies are needed to verify accuracy of axillary staging using second SLNB and also its oncologic safety.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-33

Title: Non-sentinel lymph nodes involvement in early breast cancer patients: Performance of two predictive nomograms integrating the analysis of sentinel nodes by one step nucleic acid amplification in a cohort of 299 patients

Bordes V, Campion L, Jezequel P, Lefrancois A, Boiffard F, Brillaud-Meflah V, Dravet F, Jaffre I and Classe J-M. ICO Rene Gauducheau, Nantes, France.

Body: Backgrounds: Sentinel lymph node (SLN) biopsy is a highly accurate predictor of axillary status and has become the surgical axillary standard in breast cancer patients. About 50–70 % of patients with involved SLN have no additional non sentinel node (NSN) involved, suggesting that it be possible to avoid ALND in selected patients. Many tools have been developed to help surgeons in NSLN evaluation but they all need pathological data from tumor and SLN and can't be used during surgery. Developed for intraoperative detection of SLN macro or micrometastasis involvement, the semi-automated molecular one step nucleic acid amplification (OSNA), as accurate as pathology, is available. Two simple nomograms have been developed to predict NSN involvement based on the number of CK19 mRNA copy determined by OSNA:

- Nomogram developed by Peg V (Eur J Surg Oncol 2013): based on total tumoral load (TTL). TTL is defined as the addition of CK19 mRNA copies of each positive SLN (copies/ μ L). A $TTL \geq 1.2 \times 10^5$ copies/ml (specificity=85.3%, negative predictive value (NPV) = 80%) can predict NSN involvement.

- Nomogram developed by Di Filippo F (Journal of Experimental & Clinical Cancer Research 2015): based on the number of CK19 mRNA copies and ultrasound tumor size. These two variables are categorized using quartiles with a score for each and the addition of both corresponds to a probability of NSN involvement (sensitivity = 98.1%, NPV = 92.5 %).

Patients and Methods: this is a retrospective study of 299 patients. Each patient had SLN involvement (macro or micrometastasis) and underwent a complementary ALND. The main objective was to evaluate the performance of each nomogram using a discrimination ability model, assessed by ROC analysis. Predictive accuracy was measured by the area under ROC curves (AUC) reported with its 95 % confidence interval. The second objective was to compare the two nomograms using Hanley & McNeil method, to test the statistical significance of the difference between the AUC. Analysis was performed using stata 13.1 SE.

Results: The mean age was 59, 1 year. Most patients were treated for an infiltrating ductal carcinoma (80.3%, 240/299). The mean ultrasound tumor size was 13 mm and the mean pathological tumor size was 15 mm. The median number of examined SLN was 2 with a macro-metastasis in 67, 6%, 202/299). 70 patients had involved nodes in ALND (23%).

The discrimination of N Peg, quantified with AUC was 0.685 ($p < 0, 00001$). The discrimination of N Di Filippo, quantified with AUC was 0.72 ($p < 0, 00001$).

Hanley & McNeil method shows that Di Filippo nomogram is significantly superior to Peg nomogram ($p = 0,048$).

Conclusion: The current study shows that these two nomograms are reliable and can be used to predict NSLN involvement. The combination of molecular data and ultrasound tumor size seems to be more efficient than molecular data alone. These results are similar to results of nomogram studies based on pathological analysis but only these nomograms integrating molecular data can be used during the surgery.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-34

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-35

Title: A one-day protocol with activities lower than 20 MBq for the detection of sentinel lymph nodes - Experience after 150 cases

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Body: Introduction:

Common protocols for the detection of sentinel lymph nodes (SLN) in early breast cancer often include injection of the tracer one day before surgery. In order to detect enough activity on the day of surgery, the applied activity in many protocols is as high as several hundred MBq. Even in common one-day protocols the activity applied is often up to 50 MBq. We developed a one-day protocol with a mean activity lower than 20 MBq in order to reduce radiation exposure for patients and staff. Here we are presenting our experience after 150 cases.

Material and methods:

150 patients with clinically and sonographically negative axilla (cN0) and no multicentricity underwent a SLN biopsy using a low-dose protocol performed on the day of surgery. After ultrasound-guided injection of the tracer (Technetium99) lymphoscintigraphy was performed in all cases. 7 minutes before the first cut 5 ml blue dye was injected in the region of the areola. Fresh-frozen sections of the SLN(s) were not performed.

Results:

In 149 of 150 patients (99.3%) at least one SLN could be identified by lymphoscintigraphy. The detection rate during surgery with combined tracers Technetium99 and blue dye was 100%. The mean applied activity was 17.8 MBq (9-20). A mean number of 1.3 (0-5) SLNs were identified by lymphoscintigraphy, a mean number of 1.7 (1-5) SLNs were removed during sentinel lymph node biopsy. 36 patients received a secondary axillary dissection according to the historical standard because of involved SLNs. In 11 cases (30.6%) additional involved lymph nodes were found.

Conclusion:

One-day protocols with an activity lower than 20 MBq are a safe alternative to two-day protocols with significantly higher radiation doses. Using Technetium99 and blue dye in a dual tracer approach, detection rates of 100% are possible in clinical routine with minimal radiation exposure for patients and staff. The number of removed lymph nodes, the rate of secondary axillary dissections and the number of cases with additional involved lymph nodes is not higher than in published trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-36

Title: Ex vivo shear-wave elastography of axillary lymph nodes predicting nodal metastasis in patients with primary breast cancer: A pilot study

Lim JW, Lee HW, Park JT, Ahn SG and Jung J. Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea.

Body: Objective: To evaluate the feasibility of shear-wave elastography using breast ultrasonography in identifying metastasis of removed sentinel lymph nodes during the operation for treatment of breast cancer.

Background: Conventional method for identification of sentinel nodal metastasis is time and cost consuming. The optimal method for identification of nodal status is important.

Methods: Excised sentinel lymph nodes during the operation were prospectively examined with the elastography. Metastatic status of lymph nodes was confirmed with permanent histology. Only macrometastasis was regarded as positive. Elastic values measured by the ex vivo elastography and nodal characteristics were analyzed to correlate with nodal metastasis.

Results: A total of 274 lymph nodes harvested from 68 breast cancer patients at Gangnam Severance Hospital from May 2014 to April 2015 were included this study. There was the difference of elastic values between nodes with and without metastasis (mean stiffness, 41.6 kPa and 17.4 kPa, $P < 0.001$). Mean sizes of metastatic nodes (range 0.36-2.59 cm) were significantly larger than that of non-metastatic nodes (1.0 cm versus 0.75 cm, $P < 0.001$). Moreover, there was a correlation between the size of metastatic nodes which ranged from 0.7 to 21.5 mm with a median of 7 mm and nodal stiffness (correlation coefficient of mean stiffness, $r = 0.431$). The area under the receiver operating characteristic curve (AUC) by the mean stiffness was 0.794. The combination of size of nodes, mean stiffness and ratio made AUC of 0.856.

Conclusions: In our study, ex vivo shear-wave elastography of sentinel lymph nodes was a feasible method to predict metastasis. Through the validation study, ex vivo elastography could be helpful to determine metastasis of sentinel lymph nodes during the operation.

Keywords Breast cancer; Elastography; Lymph node metastasis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-37

Title: Technical feasibility and validity of sentinel lymph node biopsy in patients with ipsilateral breast tumor recurrence

Matsumoto A, Jinno H, Yanagisawa T, Yoshikawa M, Takahashi Y, Seki T, Takahashi M, Hayashida T, Ikeda T and Kitagawa Y. Teikyo University School of Medicine, Tokyo, Japan; Keio University School of Medicine, Tokyo, Japan and Kitasato University Kitasato Institute Hospital, Tokyo, Japan.

Body: Background: The incidence of ipsilateral breast tumor recurrence (IBTR) was reported to be approximately 5-10% of breast cancer patients who had breast-conserving surgery. However, the role of sentinel lymph node biopsy (SLNB) in patients with IBTR still remains to be elucidated. The aim of this study was to evaluate feasibility and validity of sentinel lymph node biopsy for ipsilateral breast tumor recurrence (second SLNB).

Patients and methods: A prospective database of 1607 patients with clinically node-negative breast cancer who underwent SLNB from January 2005 to May 2015 was analyzed and 46 patients with IBTR underwent SLNB. Lymphatic mapping was performed using a combined method of blue dye and radioisotope. ICG fluorescence imaging was performed in cases with failure of identification by blue dye and radioisotope.

Results: The median age was 52 (range: 36-82) years at the time of second SLNB and the mean size of recurrent tumor was 1.39 ± 0.63 cm. Thirty-one (67.4%) and 10 (21.7%) patients had a history of previous SLNB and axillary lymph node dissection (ALND), respectively. Another five (10.9%) patients had no previous axillary surgery for primary tumors. Preoperative lymphatic mapping by lymphoscintigraphy was successfully performed in 24 of 36 patients (66.7%). The identification rate by lymphoscintigraphy among patients with previous SLNB, ALND, and no axillary surgery was 64.0% (16/25), 66.6% (6/9) and 100% (2/2), respectively ($P=0.583$). Overall, sentinel lymph nodes (SLNs) were successfully identified in 37 (80.4%) of 46 patients during surgery. The identification rate in patients with previous SLNB, ALND and no axillary surgery was 80.6% (25/31), 80.0% (8/10) and 80.0% (4/5), respectively ($P=0.990$). The aberrant lymphatic drainage to extra-ipsilateral axilla was found more frequently in patients with previous ALND compared with previous SLNB and no axillary surgery (40.0% vs. 6.5% vs. 0%, $P=0.015$). Among three (6.5%) patients with SLN metastases, one patient with previous SLNB had macrometastasis at the ipsilateral axilla and ALND found a positive non-SLN (1/21). The remaining two patients with previous SLNB and ALND had micrometastases at ipsilateral and contralateral axilla, respectively and both patients underwent no further axillary treatment. After second SLNB, systemic treatment including chemotherapy, endocrine therapy and trastuzumab was performed in 17 (37.0%), 36 (78.3%) and seven (15.2%) patients, respectively. No axillary recurrence was observed after a median follow-up time of 37.2 months from surgery for IBTR.

Conclusions: Second SLNB is technically feasible regardless of types of previous axillary surgery and may avoid complications from unnecessary ALND for IBTR. Furthermore, it could improve risk prediction for IBTR and provide valid information for deciding adjuvant therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-38

Title: Factors that can be used to guide further axillary treatment following positive sentinel lymph node biopsy

McIntyre N, Murray J, FitzGerald SC C, Murphy DS S and Lannigan AK K. Lanarkshire Breast Unit, Wishaw, Lanarkshire, United Kingdom.

Body: Aims: Sentinel lymph node biopsy (SLNB) is used to stage the axilla in breast cancer patients with clinically and radiologically normal lymph nodes. The burden of involved lymph nodes in a SLNB sample can be calculated using the formula: Total number of positive sentinel nodes / Total number of sentinel nodes= Positive SLN Ratio (PSLNR). This study looked at the value of using PSLNR to select those patients who may benefit most from further axillary treatment.

Methods: Data from all patients who underwent axillary ultrasound and SLNB in NHS Lanarkshire under the care of four consultant surgeons from January 2010-June 2015 was collected and recorded prospectively.

Results: 799 patients were identified and those who had an upfront sentinel node biopsy before neoadjuvant chemotherapy were excluded leaving a sample group of 748 patients. 182 patients had a positive SLNB (24%).. Of the 114 patients who had an axillary lymph node clearance (ALNC) following positive SLNB, 46 had further involved lymph nodes (40%). 70% of patients with further involved nodes on ALNC had a high PSLN ratio of >0.5 on initial SLNB, but only 47% of patients who had no further positive nodes on ALNC, had a high PSLN ratio of >0.5 (p=0.014). 21% of non-surgically managed patients (who had either radiotherapy or no further axillary treatment) had a ratio >0.5. Of those patients who had a positive SLNB, 9.9% had an abnormal axillary US with a normal pre-operative biopsy compared to 7.7% of abnormal scans in patients with a negative SLNB.

Conclusions: The majority of patients who underwent ALNC following a positive SLNB did not have any further nodal disease and could have been managed without further surgery. The PSLN ratio can be useful together with other pathological factors to help select those who could potentially be spared further axillary treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-01-39

Title: Axillary reverse mapping using fluorescence imaging in the surgical treatment for breast cancer patients

Liu J, Li J, Zhou J, Cui H, Xiang J, Jia P, Xiang A and Chen H. Affiliated Hangzhou Hospital of Nanjing Medical University, Hangzhou, Zhejiang, China.

Body: Background: Axillary lymph node dissection (ALND) is the standard surgical treatment for breast cancer patients who have axillary nodal metastasis but do not meet the criteria of Z0011 trial. Arm lymphedema is one of the most common and serious complications of ALND. Based on the hypothesis that the lymphatic system of the upper extremity may be separate from that of the breast, the axillary reverse mapping (ARM) technique has been developed to identify the axillary lymph nodes and lymphatics that receive the lymph from the upper limb, and to preserve them during ALND in order to prevent arm lymphedema. However, it is difficult to keep the arm lymphatic system intact because of the lack of full understanding of anatomical variations of the ARM nodes and lymphatics in the axilla, the inconsistency of different ARM techniques in identifying the axillary ARM nodes, and the potentially high rate of ARM nodal metastases in breast cancer patients. The aims of this study was to know (1) the identification rate of ARM with fluorescence imaging (2) the distribution of these ARM nodes and (3) whether it's safe to spare the ARM nodes in breast cancer patients during SLNB or ALND.

Method: Forty female patients of primary invasive breast cancer between 28 to 69 years old were included in the prospective study. Patients who had bilateral breast cancer, the history of axillary surgery, or the history of neoadjuvant chemotherapy were excluded. Methylene blue dye was used in the SLNB procedure. The ARM procedure was carried out to all the patients with a subcutaneous injection of the fluorescent dye indocyanine green into the medial bicipital sulcus in the upper arm about 15 minutes before SLNB or ALND. During surgery, photodynamic eye was used to detect the fluorescent nodes in the axilla. The fluorescent ARM nodes in the surgical region were removed and sent for pathological examination of node metastasis.

Results: ARM was successful in 8 out of the 35 patients who underwent SLNB + ARM, in 3 out of the 5 patients who underwent ALND + ARM and in 25 out of the 35 patients who underwent ALND following SLNB + ARM. The ARM identification rate was 22.9%(8/35) in SLNB, and was 93.3%(28/30) in ALND. Totally, 119 fluorescent ARM nodes were detected, and 57.1 % (68/119) of them were located under the axillary vein, above the 2nd intercostobrachial nerve, and in the lateral of thoracic dorsal neurovascular bundle. Six out of 35 patients were found to have ARM-SLN crossover during SLNB+ARM procedure. Two out of 24 patients were found to have the ARM nodal metastases, with the pathological node stage being pN2, pN3 respectively.

Conclusion: ARM with fluorescence imaging using indocyanine green was sensitive for identification of ARM nodes in ALND with a high success rate; ARM nodes had a certain distribution pattern in the axilla; ARM should not be performed to the patients with multiple nodal metastases in the axilla.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-02-01

Title: Identifying the remaining causes of hereditary breast cancer

Campbell IG G, Trainer AH H, Devereux L, James PA A, Rowley S and Li N. Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia.

Body: Identifying the missing hereditary factors of familial breast cancer could have a major and immediate impact on reducing breast cancer risk in these family members.

Up to 1,325 candidate breast cancer predisposition genes, identified through exome sequencing of BRCAx families, were sequenced in index cases of up to 4,000 BRCAx families and 4,000 cancer free women from the LifePool study in Australia. Interrogation of the data to refine the highest priority candidates is ongoing, but it is noteworthy that known (PALB2) or suspected (MRE11A) moderately penetrant breast cancer genes showed enrichment of loss of function (LoF) mutations in this dataset. Conversely, some other recently proposed breast cancer genes (BRIP1 and RINT1) did not show a significantly higher LoF mutation frequency in the cases compared to controls. Based on the number of LoF mutations leading candidates include NTHL1 (12 cases versus 4 controls) and ALKBH1 (7 cases versus 2 controls) which are each important members of the base excision repair and direct nucleotide repair pathways. We examined other genes in the base excision and direct repair pathways that were on our sequencing capture design and observed a significant enrichment of potentially deleterious mutations in 12 genes (NTHL1, OGG1, APEX1, APEX2, NEIL1, NEIL2, NEIL3, MUTYH, MPG, ALKBH1, ALKBH2, ALKBH3): Among the 1,638 cases and 1,654 controls analysed to date, 76 LoF variants were detected in these genes among the cases versus 47 LoF variants among the controls ($p=0.007$). Based on the overall distribution of variants between cases and controls the probability of selecting 12 genes with such enrichment from the 1,325 genes screened was less than 1 in 200.

Our data implicates rare mutations in base excision and direct DNA repair pathways genes as moderate-penetrance breast cancer susceptibility alleles.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-02-02

Title: Clinical significance of TP53 mosaic mutations uncovered in constitutional genetic testing

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Body: Background: Mosaic findings (mutations outside of the expected 50:50 allele ratio) are often detected in the course of routine constitutional genetic testing due to the high sensitivity of next-generation sequencing (NGS) technologies. The significance of these findings is however often unclear. They can result from constitutional mosaicism secondary to an early embryological event, an acquired somatic mutation in hematopoietic cells, a technical artifact, or (less likely) a reversion event. Studies have shown that clonal hematopoiesis increases with age, seen in up to 10% and 25% of individuals 65 and 90 years of age, respectively. Additional studies have demonstrated expansion of TP53 mutations in peripheral blood cells following chemotherapy administration. We therefore wanted to investigate the potential clinical significance of mosaic mutations found in the peripheral blood in samples submitted for hereditary cancer testing with a focus on TP53.

Methods: We examined high-depth NGS data from blood cell-derived DNA for mutations with pathogenic potential suggestive of mosaicism (5-30% allele frequency) in known hereditary cancer genes. For each gene, we determined the average ages of patients tested and compared these distributions to the distribution of ages of patients found to have mosaic mutations. For the mosaic TP53 mutations, we further characterized the mutations found as well as described the clinical scenarios in which the mosaic findings occurred.

Results: To date, 101 patients were found to have possibly mosaic mutations with pathogenic potential, including 26 cases with TP53 mutations (0.07% of patients tested), 16 with CHEK2 mutations (0.05%), and 16 with ATM mutations (0.05%). Significant bias towards older ages is seen in patients with ATM ($p=0.0026$) and CHEK2 ($p=4.2 \times 10^{-6}$) mosaic findings. Mosaic TP53 and NF1 mutations were also enriched in older patients, although not as significantly as CHEK2 and ATM mosaic patients. 24/25 patients with TP53 mosaic mutations with a known history were cancer affected. In the COSMIC database, 20/20 of the TP53 mosaic mutations are associated with neoplastic conditions and 13/20 are associated with hematolymphoid neoplasms. 15/20 of the pathogenic mosaic TP53 findings were seen previously in our lab at a ratio corresponding to germline, thereby significantly lessening the possibility of technical artifact.

Conclusion: The statistically significant older ages of patients with ATM and CHEK2 mosaic variants suggests that many of these are acquired changes in hematopoietic stem cells rather than constitutional mosaicism per se. Although NF1 is known to be associated with constitutional mosaicism, the bias towards older patients of the NF1 mosaics we saw similarly suggests that most are acquired changes in the hematopoietic stem cells. The less significant age bias in the TP53 events suggests that chemotherapy administration may be leading to enrichment of TP53 mutated clones, although an increased rate of constitutional mosaicism cannot be ruled out.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-02-03

Title: Optimized prediction of deleterious missense mutations in *BRCA1* and *BRCA2* genes

Hart SN N, Hoskin T, Shimelis H, Feng B, Lindor NM M, Monteiro A, Iversen E, Goldgar DE E, Suman V and Couch FJ J. Mayo Clinic, Rochester, MN; University of Utah, Salt Lake City, UT; Mayo Clinic, Jacksonville, FL; Moffit Cancer Center, Tampa, FL and Duke, Durham, NC.

Body: Approximately 15% of genetic screens for mutations in *BRCA1* and *BRCA2* identify Variants of Uncertain Significance (VUS). Primarily missense mutations, VUS are often difficult to interpret, leading to either uncertainty in how to properly counsel a patient or an unnecessary prophylactic surgery. Given the paucity of data for which missenses are classified as truly pathogenic, computational deleterious missense prediction (DMP) algorithms are used to predict whether a mutation is likely deleterious or neutral. Accuracy of DMPs can vary considerably and have only been calibrated on a relatively small number of missense mutations of demonstrable effect on protein function. In this study, the performance of 41 different DMPs was compared to functional data from 455 functionally characterized missense variants in *BRCA1* and *BRCA2*. New optimized thresholds for classifying missense mutations as deleterious are presented for several existing models as well as a newly derived naïve voting method (NVM). The areas under the curve estimates for the NVM approach are between 0.889-0.922, much higher than previous methods. We estimate that the overall pathogenic potential of missense variants to be 6.8% for *BRCA1* and 3.2% of *BRCA2*, but can be as high as 50% depending on protein location. Overall these results provide key insights into how to predict deleterious missense mutations in *BRCA1* and *BRCA2*.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-02-04

Title: Li-Fraumeni syndrome in females with early onset breast cancer in a Mexican population

Gallardo-Alvarado LN N, Cantu-De Leon DF F, Tusie-Luna T, Tusie-Luna I, Diaz-Chavez J, Herrera EM M, Chavez-MacGregor M, Bargallo-Rocha E, Villarreal C, Herrera.Montalvo LA A and Segura-Kato YX X. Instituto Nacional de Cancerologia, Mexico, Mexico; Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán"/UNAM, Mexico, Mexico; University of Texas MD Anderson Cancer Center, Houston, TX and Genyka Diagnostico S.A.P.I de C.V, Mexico, Mexico.

Body: Background: Breast cancer in Mexican patients presents in average 10 year earlier compared to other populations. Among patients with *TP53* mutations, breast cancer is the most common malignancy. In this study we aim to determine the rate of *TP53* mutations among young Mexican breast cancer patients. **Methods:** Breast cancer patients younger than 45 were retrospectively identified, only patients without BRCA1/2 mutations were included. Clinical records and pedigrees were reviewed. Next generation sequencing test for *TP53* was performed from blood specimens. All pathogenic mutations were confirmed by Sanger sequencing and verified if were included in the IACR TP53 database. **Results:** Among the 78 patients younger than 45yo evaluated and tested with next generation sequencing, we identified 5 patients with a *TP53* mutation (6.4%). All were younger than 36 corresponding to a rate of 9.4% of mutations in this group of individuals (n = 53). Two, not previously described, frameshift mutations were found (c.291delC and c.273dupG) and three missense mutations (c.844C>T, c.517G>A, c.604C>T). A VUS c.672G>A that causes a silent mutation in a splicing-donor site was identified. All patients with a *TP53* mutation had locally advanced disease and tumors were high grade and Her2 positive. **Conclusions:** Among Mexican women younger than 45yo, the rate of *TP53* mutations was higher than in other populations where it's estimated to be 1%. Among patients younger than 36 the proportion was even higher. All patients had a family history suggestive of Li-Fraumeni syndrome. The VUS found in our series needs a deeper analysis (family segregation, structure and function of the resulting protein). Our results support the international recommendation of performing molecular testing for *TP53* in breast cancer patients younger than 35 years. Identification of patients with *TP53* mutation has important prognostic, therapeutic and quality of life implications for the proband in addition of the risk reduction strategies and intensive surveillance recommended in their families.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-02-05

Title: Comparison of non-breast and ovarian cancer phenotypes of *BRCA1/2* mutation carriers across multi-gene panels

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Body: Background: *BRCA1/2* germline mutations account for the majority of hereditary breast and ovarian cancers. Until 2013, the only way to identify individuals with BRCA mutations was through single gene testing. With multi-gene panel testing (MGPT) including *BRCA1/2*, BRCA mutations are being identified at an increased rate. To date, the phenotype of *BRCA1/2* mutation carriers includes an increased prevalence of breast, ovarian, prostate, and pancreatic cancer, as well as melanoma. The phenotype, however, of mutation carriers identified by panel testing is not well understood.

Methods: All sequential cases submitted to our laboratory for MGPT including *BRCA1/2* between June 2013 and June 2015 and *BRCA1/2* single gene testing between June 2013 and February 2015 were retrospectively reviewed. Data from 77,345 test request forms were reviewed; probands with a *BRCA1* or *BRCA2* mutation were selected and analyzed. Probands with no personal history of cancer/not provided (n=528) were excluded.

Results: Of 2,967 *BRCA1/2* positive probands, 2,439 (82.2%) had a personal history of cancer, with 2,794 cancers reported. On all tests completed, with the exception of a pancreatic cancer focused panel, breast and/or ovarian cancer were the most commonly observed cancer types (n=2,364, 84.6%). On single gene testing (n=739), prostate (n=16, 2.2%), colorectal (n=8, 1.1%), and pancreatic (n=7, 0.9%) cancers were the other most frequent cancers reported. Across all MGPT cases (n=1,700), additional observed cancers included uterine (n=48, 2.8%), colorectal (n=46, 2.7%), pancreatic (n=37, 2.2%), melanoma (n=29, 1.7%), and thyroid (n=27, 1.6%). For breast cancer-specific gene panel cases (n=934), additional cancers included thyroid (n=12, 1.3%) and uterine cancer (n=10, 1.1%). For gynecologic cancer gene panel cases (n= 482), additional cancers included uterine (n=21, 4.4%), colorectal (n=11, 2.3%), and melanoma (n=11, 2.3%). Cases tested via a pancreatic cancer gene panel (n=25,) had pancreatic cancer (n=17, 68%) reported most frequently, followed by breast (n=6, 24%), colorectal (n=3, 12%), and melanoma (n=3, 12%). For comprehensive cancer gene panel cases (n=254), colorectal (n=28, 11.0%), uterine (n=17, 6.7%), pancreatic (n=14, 5.5%), prostate (n=10, 3.9%), thyroid (n=10, 3.9%), and a variety of rare tumor types (n=15, 5.9%) were among the additional cancers reported.

Discussion: As expected, the majority of cancers reported on almost all tests were breast and/or ovarian cancer. More specifically, the observed cancers for tumor specific cancer panels, matched the relevant cancer type (i.e. pancreatic cancer most commonly reported in those tested via a pancreatic cancer panel). However, on the comprehensive cancer panels, other than breast and ovarian cancer, there appears to be a more even distribution of various cancer types, including rare tumors, in probands who have a *BRCA1* or *BRCA2* mutation. Further studies should be conducted to examine the phenotypes of BRCA mutation carriers identified via comprehensive cancer panels to determine the association and prevalence of unexpected cancer types with *BRCA1/2* mutations. This could have important implications for determining the most appropriate genetic test and management of BRCA mutation carriers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-02-06

Title: Genetic counseling, germline genetic testing, and impact of results in patients with newly diagnosed breast cancer

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Body: The surge in *BRCA1/2* and multiple-gene panel testing after a diagnosis of breast cancer has fueled concerns about how genetic testing results will be integrated into patient management. However, there is virtually no research about the timing or extent of genetic counseling before or after testing or the impact of genetic results on bilateral mastectomy (BLM) use since the advent of more widespread testing. **Methods:** A population-based sample of 3600 patients newly diagnosed with breast cancer identified by two SEER registries (Georgia and Los Angeles County) were sent surveys two months after surgery (Dx dates 2014-15) about their genetic testing and treatment experiences. Survey information was merged with SEER data. We examined patterns and correlates of counseling and genetic testing and the impact of results on patient preferences for BLM and receipt of BLM. **Results:** Among 2388 patients with unilateral breast cancer (response 70%), 697 (29.2%) had elevated pre-test risk of a germline mutation (based on age, family cancer history, ancestry, and tumor subtype). One-quarter of these higher risk patients (25.6%) did not discuss whether to have testing with any provider, 26.1% discussed it with clinicians only, and 48.3% had a visit with a genetic counselor. Half of patients with elevated pre-test risk (51.2%) were tested: 6.6% before diagnosis, 65.4% after diagnosis but before surgery and 28.0% after surgery. Higher risk patients who underwent testing were younger ($p < .001$) and had higher income ($p = .029$) but rates did not differ significantly by race, education, insurance, marital status, cancer stage, comorbidities, or geographic site after controlling for all covariates. There was wide variation in the type of professional who discussed test results with patients: discussed with surgeon only (17.8%), medical oncologist only (19.7%), both physicians but no counselor (4.8%), or genetic counselors (56.8%). Among all testers in the total sample ($n = 667$), 54 (9.4%) reported a pathogenic mutation (12.1% of higher risk patients vs 5.7% of low risk patients) and 59 (10.0%) reported a variant of unknown significance (VUS) (10.2% of higher risk patients vs 9.9% of lower risk patients), $p = .027$ for differences between groups. Two-thirds (60.4%) of patients with pathogenic mutations reported that the test made them more interested in BLM vs 8.8% of those with a VUS, and 11.4% of those with negative tests, $p < .001$. Two-thirds (69.2%) of those with pathogenic mutations received BLM vs 21.9% of those with VUS and 27.9% of those with negative tests, $p < .001$. **Conclusions:** Many patients newly diagnosed with breast cancer at higher risk of carrying a pathogenic mutation do not receive pre-test counseling or genetic testing and disparities are observed. There is wide variability in the timing of genetic testing after diagnosis and with which clinician the findings are discussed. Taken together, these results suggest that germline genetic testing after a diagnosis of breast cancer is poorly integrated into practice. However, the impact of genetic test results on patient attitudes and receipt of bilateral mastectomy suggests that genetic testing does help target prevention to a patient's future risk for a new primary breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-02-07

Title: Predicting germline mutations in *BRCA1/2* and beyond: A comparison of women with single and multiple breast primaries

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Body: Synchronous or metachronous breast primaries are a well-known indication of hereditary breast cancer, particularly within *BRCA1/2* mutation carriers. However, the frequency of gene mutations within this patient group has not been well defined, especially in the setting of multi-gene panel testing (MGPT). We conducted a retrospective review of mutation carrier status in a population of females with breast cancer(s), but no other reported cancer diagnoses, and who had MGPT at a single diagnostic laboratory. Among 31,864 females tested, the following were excluded from analysis: 5389 (17%) had variants of unknown significance (VUS), 133 (0.4%) had moderate risk mutations and 316 (1.0%) had *MUTYH* monoallelic mutations. For the remaining 26,026 females, we evaluated whether mutation status is associated with risk of multiple breast primaries using Fisher's exact test and logistic regression analysis adjusting for age at testing, age at first breast cancer diagnosis, and mutations in other genes. The number of genes analyzed ranged from 5-49, depending on the panel ordered. Gene-specific analyses were limited to with 10 or more mutations in this cohort (*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CDKN2A, CHEK2, MRE11A, MUTYH, MSH6, NBN, NF1, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, and TP53*).

In this cohort the average age of first breast cancer diagnosis was 47.7 (range 12-95) and the average age of second diagnosis was 56 years (range 17-89). A positive result (pathogenic mutation or variant, likely pathogenic) in any gene was more likely for women with three or more breast cancer primaries ($p=0.007$) and two or more primaries ($p=1.2e-08$) than those with one breast primary.

Overall, women with a mutation in any gene were more likely to have multiple primary breast cancers than those without mutations. Specifically, women with mutations in *ATM, BRCA1, CDH1, PALB2, PTEN, and TP53* mutations were more likely to have multiple breast primaries than non-carriers of mutations in those genes (table 1).

Table 1: Risk of multiple breast primaries in mutation carriers versus non-carriers

Gene	No. (%) with multiple primaries		OR (95% CI)	p-value
	Carrier	Non-Carrier		
ATM	55/262 (21%)	2189/15057 (15%)	1.6 (1.1, 2.2)	0.006
BRCA1	100/526 (19%)	3104/24117 (13%)	1.9 (1.5, 2.4)	3.2e-07
CDH1	6/24 (25%)	3338/25016 (13%)	2.8 (1.0, 1.7)	0.04
PALB2	45/218 (21%)	2199/15101 (15%)	1.7 (1.2, 2.4)	.004
PTEN	7/28 (25%)	3429/25788 (13%)	3.8 (1.5, 8.9)	0.003
TP53	19/96 (20%)	3419/25787 (13%)	2.4 (1.3, 4.0)	0.002

Our results show that women with multiple breast primaries are more likely to have mutations in some genes than others. Interestingly, all genes with significant odds ratios are well-described and most are known to cause high risk for breast cancer, with the exception of *ATM*. Additional studies are needed to confirm these results and quantify risks for second primary breast cancers. With further work defining the risks of multiple primary breast cancers, this information could be implemented into clinical practice to aid women in risk management following a positive result.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-03-01

Title: Mutational landscape of breast cancers from PALB2 germline mutation carriers

Burke KA A, Berman S, Geyer FC C, Piscuoglio S, Ng CK K, Wen YH, Mannermaa A, Peterlongo P, Tondini C, Janatova M, Soo Hwang T, Ng P-S, Looi LM, Chenevix-Trench G, Southey MC C, Weigelt B, Foulkes W, Tischkowitz M, Reis-Filho JS S and PALB2 Interest Group. Memorial Sloan Kettering Cancer Center, New York, NY; University of Eastern Finland, Kuopio, Finland; IFOM Fondazione Istituto FIRC di Oncologia Molecolare, Milan, Italy; Ospedale Papa Giovanni XXIII, Bergamo, Italy; Charles University, Prague, Czech Republic; Cancer Research Malaysia, Selangor, Malaysia; University of Malaya, Kuala Lumpur, Malaysia; University of Queensland, Brisbane, Australia; University of Melbourne, Melbourne, Australia; McGill University, Montreal, Canada and University of Cambridge, Cambridge, United Kingdom.

Body: Background: The PALB2 gene encodes the partner and localizer of the BRCA2 protein, which participates in homologous recombination during DNA repair via an interaction with BRCA1 and BRCA2. Germline mutations in PALB2 are associated with an increased risk of breast cancer, with a cumulative risk of 35% by age 70 in female PALB2 mutant carriers. The aims of this project were to characterize the genomic landscape of PALB2 breast cancers and define the differences in the repertoire of somatic genetic alterations and mutational signatures between PALB2, BRCA1 and BRCA2 breast cancers.

Methods: Representative samples from fourteen breast cancers from patients with known PALB2 germline mutations (seven frame-shift (2 H1170fs, 3 K346fs, 1 T841fs and 1 L531fs), five truncating (3 W1038* and 2 Q775*) and two missense (W1140G and L35P)) were microdissected to ensure a tumor cell content of >70%. DNA samples from microdissected tumors and their matched normal counterparts were subjected to whole exome sequencing on an Illumina HiSeq2000 to a median depth of 118x (range 33-193x). Somatic single nucleotide variations were detected using MuTect, and small insertions and deletions were identified using Strelka and Varscan2. Using ABSOLUTE and FACETS, we investigated the presence of loss of heterozygosity (LOH) of the PALB2 wild-type allele in these tumors. In addition, the mutational signatures and large scale state transitions (LSTs) were defined. The repertoire of somatic mutations identified in PALB2 breast cancers was compared to that of breast cancers from BRCA1 (n=11) and BRCA2 (n=10) germline mutation carriers from The Cancer Genome Atlas study.

Results: PALB2 breast cancers were found to harbor a median of 80 somatic mutations (range 22-286) and one somatic mutation (range 0-13) affecting known cancer genes. Somatic loss of the PALB2 wild-type allele was found in five cases, and in three additional cases, a second PALB2 somatic mutation likely constituted the second 'hit' (two with truncating mutations, Q479* and Q61*, and one with a Q921fs frameshift mutation). Six PALB2 breast cancers displayed the BRCA mutations signature; of these, five had PALB2 bi-allelic inactivation (three LOH of the wild-type allele and two a second PALB2 somatic mutation). 71% of the samples were found to have LSTs, including all cases with a BRCA mutational signature. A significant association between PALB2 bi-allelic inactivation and concurrent BRCA signature and high LST was observed (p=0.015). Breast cancers from PALB2 mutation carriers had fewer somatic TP53 mutations than BRCA1 breast cancers (3/14, 21% vs 9/11, 82%, p=0.004), but no difference in the repertoire of somatic mutations compared to that of BRCA2 breast cancers.

Conclusions: PALB2 breast cancers were found to harbor pathogenic mutations in driver genes, including TP53, PIK3CA, NF1 and NCOR1, however lacked highly recurrent somatic mutations. Unlike BRCA1/2 breast cancers, the majority of breast cancers from PALB2 germline mutation carriers lacked LOH of the PALB2 wild-type allele. Importantly, however, an association between PALB2 bi-allelic inactivation and the BRCA mutational signature and LSTs was observed, providing additional evidence for a homologous recombination-deficient phenotype at least in a subset of PALB2 cancers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-03-02

Title: Differential mutation pattern between neoadjuvant and metastatic settings in breast cancer patients

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Body: Background: Dysregulated signaling pathways occur in human cancers including breast cancer, making it a rational target for novel genome guided combinatorial personalized therapies. The aim of the present study was to investigate the different genetic mutation pattern between neoadjuvant and metastatic settings in breast cancer patients to guide research and clinical treatment.

Material and Methods: 150 breast cancer patients were involved in this study. 38 patients were receiving neoadjuvant treatment and 112 patients were in the metastatic setting. Tumor specimens obtained from the 150 patients were subjected to genetic mutation testing by FoundationOne. Genetic alterations detected by FoundationOne test were collected and analyzed.

Results: 96 and 149 different genes were reported by FoundationOne in neoadjuvant and metastatic setting respectively. The average number of non-synonymous mutation was five per case in the neoadjuvant setting and six per case in the metastatic setting. TP53 (58%), MYC (32%), PIK3CA (29%), PTEN (16%), CDH1 (13%), CCND1 (11%), EMSY (11%), LYN (11%) and ZNF703 (11%) were the most seen mutations in neoadjuvant setting. TP53 (40%), PIK3CA (39%), MYC (22%), CCND1 (21%), FGF19 (21%), FGF4 (21%), CDH1 (20%), FGF3 (19%), ERBB2 (17%), ESR1 (14%), FGFR1 (14%), ZNF703 (14%), GATA3 (13%), MYST3 (11%), PTEN (11%), EMSY (10%), NF1 (10%) and ZNF217 (10%) were the most seen mutations in metastatic setting. ESR1 and GATA3, which are seen in 14% and 13% of metastatic breast cancer patients, were not reported in neoadjuvant breast patients. Moreover, among the 16 metastatic breast cancer patients who has ESR1 mutation, 9 (56%) of them presented with PIK3CA or other genetic mutations which are directly involved in the phosphoinositide 3-kinase (PI3K)/AKT pathway.

Conclusion: A significantly more mutation in Receptor Tyrosine Kinases (RTKs)/ Growth Factor Signaling (especially ERBB and FGFR pathways) was reported in the metastatic setting compare to the neoadjuvant setting, suggesting a critical role of the RTKs in metastatic breast cancer patients. The coexisting of ESR1 and PI3K/AKT pathway alteration and the absence of ESR1 in neoadjuvant setting also suggested that in early stage breast cancer patients who have a PI3K pathway alterations; there is a higher chance to develop ESR1 mutation with disease progression.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-03-03

Title: Molecular differences between screen-detected and interval breast cancers are largely explained by PAM50 subtypes

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Body: Purpose: Interval breast cancer is of clinical interest as it exhibits an aggressive phenotype and evades detection by screening mammography. A comprehensive picture of somatic changes that drive tumors to become symptomatic in the screening interval can improve understanding of the biology underlying these aggressive tumors.

Experimental design: Initiated in April 2013, Clinical Sequencing of Cancer in Sweden (Clinseq) is a scientific and clinical platform for the genomic profiling of cancer. The breast cancer pilot study consisted of women diagnosed with breast cancer between 2001-2012 in the Stockholm/Gotland regions. A subset of 318 breast tumors were sequenced, of which 113 were screen-detected and were 60 interval cancers. We applied targeted deep-sequencing of cancer-related genes, low-pass whole-genome sequencing and RNA-sequencing technology to characterize somatic differences in the genomic and transcriptomic architecture by interval cancer status. Mammographic density and PAM50 molecular subtypes were considered.

Results: In the crude analyses, *TP53*, *PPP1R3A*, and *KMT2B* were significantly more frequently mutated in interval cancers than in screen-detected cancers. Acquired somatic copy number aberrations with a frequency difference of at least 15% between the two groups included gains in 17q23-q25.3 and losses in 16q24.2. Gene expression analysis identified 447 significantly differentially expressed genes, of which 120 were replicated in an independent microarray dataset. After adjusting for *PAM50*, most differences were no longer significant.

Conclusions: Molecular differences by interval cancer status were observed, but they were largely explained by PAM50 subtypes. This work offer new insights into the biological differences between the two tumor groups.

Translational relevance: Although screen-detected cancers are biologically distinct from interval cancers in terms of somatic mutations, copy number aberrations and gene expression, most of the differences are no longer significant after adjusting for breast cancer intrinsic subtypes (PAM50). We also show that the molecular differences appear to form a spectrum from less aggressive (screen-detected) to more aggressive (interval) manifestations of the disease, which can be characterized by PAM50 subtypes, namely, luminal A, luminal B, HER2-enriched and basal-like, in that order. This work clarifies the picture on what type of breast cancer we are likely to identify through population-based screening, and what type of cancer we are likely to miss. Current knowledge of PAM50 subtype-specific risk factors need to be expanded as our findings might influence how we screen women with a higher risk of basal-like breast cancer for example, beyond known risk groups *BRCA1* mutation carriers and women of African-American descent.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-03-04

Title: Application of digital-PCR technology to determine c-MET copy number variation in paired primary breast cancer and brain metastases

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Body: INTRODUCTION:

c-MET amplification/overexpression has been associated with treatment failure and progression in many cancers, including breast cancer (BC). c-MET showed amplification by fluorescent *in situ* hybridization (FISH) in 27% of trastuzumab-treated HER2-positive patients. These patients had a high trastuzumab failure rate and a shorter time to progression. Up to 50% of patients with metastatic HER2-positive disease will develop brain metastases (BM) during their disease course and in approximately one third, brain is the first site of progression. Amplification/copy number variations (CNVs) are mainly assessed by FISH whereas overexpression is assessed by immunohistochemistry (IHC). We present a PCR-based assay (digital-PCR) able to determine CNV in c-MET and HER2 in a cohort of patients with metastatic BC to the brain and demonstrate the correlation of CNV to protein expression.

METHODS:

DNA was isolated from paraffin-embedded tissues of 23 paired primary BC-BM cases. CNV was analysed by the QuantStudio™ 3D-Digital-PCR (QS3D) and real-time qPCR (both from ThermoFisher Scientific). The breast MCF7, T47D, BT474, AU565, SKBR3 and the gastric MKN45 cell lines were used as controls for the HER2 and c-MET CNV assays. Copy number per diploid genome was calculated using the absolute quantification number of FAM-labelled target and VIC-labelled RNaseP reference multiplied by 2. Cases with ≤ 2 copies are classified as normal whereas cases with > 2 were classified as amplified. The HER2 positivity of the primary BC cases was routinely assessed by IHC. The c-MET protein expression was assessed by IHC using the c-MET(3D4) monoclonal antibody (ThermoFisher Scientific).

RESULTS:

CNV in c-MET by QS3D digital-PCR was detected in 69.6% of primary BC (ER-/HER2+:2, ER+/HER2+:5, ER+/HER2-:8, Triple-negatives:5, unknown:3) as well as 69.6% of BM, whereas HER2 CNV was observed in 39.1% primary BC and 52.2% BM. In the HER2-positive cases, the prevalence of HER2 CNV was 100% in both primary BC and BM. Within these cases, c-MET CNV was 85.7% in the primary BC and 71.43% in BM. CNVs in both genes were observed in 30.4 % of all primary and 39.1% of BM. The CNV data are presented in Table 1.

CNV data for c-MET and HER2 between primary breast cancer (BC) and corresponding brain metastatic (BM) cases

c-MET CNV			HER2 CNV		
BC	BM		BC	BM	
>2: 16	>2: 12	≤ 2 : 4	>2: 9	>2: 8	≤ 2 : 1
≤ 2 : 7	>2: 4	≤ 2 : 3	≤ 2 : 14	>2: 4	≤ 2 : 10

There was a high concordance between the QS3D and qPCR data with Pearson's $R=0.74$ ($p<0.00001$).

A significant correlation between HER2 protein expression and CNV was observed (Fisher's exact test $p=0.0005$). Data will be presented on c-MET protein expression in the pair samples.

CONCLUSIONS:

The prevalence of CNV is much higher than that reported by immunohistochemistry and FISH in the literature to date, possibly

due to the sensitivity of the digital-PCR technology. The high level of c-MET CNV in primary and metastatic BC, and the concurrent CNV in both genes warrants further investigation. It also highlights the potential to use c-MET directed therapy particularly in HER2+ BC and reinforces the potential importance of precise detection methods in both the primary and metastatic setting. Analysis of a larger series is currently on-going.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-03-05

Title: Identification, clinical characteristics and treatment outcomes of somatic human epidermal growth factor receptor 2 (ERBB2) mutations in metastatic breast cancer patients

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Body: Background

Metastatic breast cancer (MBC) is seen as incurable and advances in new therapies or targets are necessary. Targeted therapies against human epidermal growth factor receptor 2 (ERBB2) (also known as HER2) in tumors with ERBB2 overexpression due to gene amplification have improved patients outcomes. Besides ERBB2 amplification, this receptor can also be altered by somatic mutations (without ERBB2 amplification) that likely drive tumorigenesis. Neratinib, an irreversible pan-HER tyrosine kinase inhibitor, has recently been demonstrated to potently inhibit breast cancers that have amplification or activating mutations in ERBB2. This is the first study that thoroughly investigates the natural history of ERBB2 mutations in MBC patients. The primary study objectives are to 1) evaluate the frequency of ERBB2 mutations in a large MBC cohort, 2) understand standard clinical, pathological and patient characteristics associated with ERBB2 mutations, 3) identify other gene mutations/aberrations that may co-occur with ERBB2 mutations, 4) characterize treatment responses and outcomes to standard therapies in patients with ERBB2 mutant vs. wild-type tumors, and 5) determine if ERBB2 mutations can be detected from plasma ctDNA samples collected at baseline (not reported here).

Patients and Methods

This retrospective study included all MBC patients (primary metastatic or developed metastases during follow-up), independent of hormone receptor or HER2 amplification status, diagnosed between January 1st 2000 and July 31st 2015 at the Multidisciplinary Breast Center of University Hospitals Leuven, and for whom sufficient tumor material was available for DNA extraction. Extracted DNA from primary breast cancer tissues were subject to targeted NGS-based sequencing, to identify single nucleotide variants, insertion, deletion, and indels within exons 8, 17, 19, 20 and 21 of the ERBB2 gene.

Results

We established and validated a research use only next-generation sequencing assay across five exons of the ERBB2 gene. 1062 MBC patients were included and so far 177 patients were successfully screened for ERBB2 mutations resulting in an occurrence of n=10 (5.6%) in this population. The remaining patients are currently being screened for ERBB2 mutations and the overall results on all described endpoints will be available at the meeting.

Conclusion

ERBB2 mutations seem more frequent in MBC than previously thought based on the general early breast cancer population. In depth analysis of this large MBC cohort evaluating clinical characteristics and standard treatment outcomes of ERBB2 mutant MBC may provide further knowledge on this breast cancer subtype that may benefit from HER2-directed therapies which are currently under investigation in clinical trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-03-06

Title: Genomic characterization of 992 primary breast cancers

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Body: Background: We studied the associations between cancer gene alterations with clinical parameters in primary breast cancer (BC) samples of patients treated in either the FinHer or the FinXX adjuvant trial. These randomized trials accrued patients using similar inclusion criteria (node-positive, or node-negative with size >20 mm and PgR-) from the same centers, and had a similar control arm (3 cycles of docetaxel (T) followed by 3 cycles of CEF; T+CEF).

Methods: Mutations of 371 cancer-associated genes and copy number alterations (CNAs) of 86 genes or chromosomal regions were analyzed using next generation sequencing from the DNA extracted from formalin-fixed BCs. In FinHer, the comparator arm to T+CEF consisted of 3 cycles w/ly vinorelbine (V) followed by 3 cycles of CEF (V+CEF), and in FinXX of docetaxel plus capecitabine (TX) followed by 3 cycles of cyclophosphamide, epirubicin and capecitabine (CEX; TX+CEX). Adjuvant trastuzumab was administered to patients with HER2+ BC in FinHer based on random allocation for 9 weeks with either T or V, and in FinXX to all patients after May 2005, usually for 1 yr.

Results: 1,014 BCs were analyzed for mutations and CNA alterations; 992 and 915 analyses were successful, respectively. 73.7% of the BCs were ER and/or PgR+ (cut-off 10%), 11.9% ER/PgR-/HER2+, and 14.7% triple-negative. 32 genes were mutated in ≥ 10 cancers, most commonly *TP53* (38%), *PIK3CA* (33%) and *GATA3* (10%); *ErbB2* was mutated in 2.0% and *ErbB3* in 1.3%. Mutations of genes associated with hereditary BC were frequent, *CHEK2* 4.8%, *BRCA2* 3.1%, *PALB2* 2.3%, *BRCA1* 1.7%. All 101 *GATA3* mutations were found in ER/PgR+ BCs, whereas *BRCA1*, *ErbB3*, *PREX2* and *PIK3R1* mutations showed the strongest associations with ER/PgR- BC. *TP53* and *ErbB3* mutations were associated with HER2-positivity, whereas no *AKT1*, *BRCA1* or *SF3B1* mutations were detected in HER2+ BC. *RB1*, *BRCA1*, *PALB2* and *TP53* mutations were associated with high Ki-67%; *MAP3K1*, *CDH1* and *CBFB* mutations with low Ki-67%. 70% of lobular cancers harbored mutated *CDH1*, whereas *TP53* mutations were rare (4.5%). Presence of *RAD50*, *PALB2*, *CHEK2* and *TP53* were significantly associated with poor recurrence-free survival (RFS) with a hazard ratio (HR) of 4.11, 2.34, 2.22 and 1.56, respectively, whereas *PIK3CA* and *GATA3* mutations with favorable RFS (HR 0.68 and 0.55). Lobular cancers with or without *CDH1* mutation had similar RFS. The most frequently amplified genes were *ErbB2* (26%), *CCND1* (17%), *RAD21* (14%) and *c-MYC* (14%). HER2+ BCs (defined by CISH or immunohistochemistry) frequently harbored amplified *ErbB2* (88%), but also amplifications of *ErbB3*, *MYB*, *WT1*, *FOXA1* and *PIK3CA* were associated with HER2+ BC. Amplifications of several genes significantly correlated with a negative ER/PgRstatus, the ductal histological type or high Ki-67%. In the FinXX trial subset patients with mutated *TP53* had unfavorable outcome when treated with T+CEF but not when treated with TX+CEX, whereas patients with mutation in one of the 11 genes involved in DNA repair had poor outcome when treated with TX-CEX, but not when treated with T-CEF.

Conclusions: Cancer gene aberrations show varying associations with the clinical and histopathological features of BC. Such molecular variations may explain in part the variations found in the efficacy of cancer drugs between clinical trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-03-07

Title: Exome sequencing of human breast cancer tissues resistant to taxanes

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Body: Background: Although taxanes are a mainstay of breast cancer treatment, some cases are resistant to these drugs. This is a crucial issue in breast cancer therapy. In the emerging era of next-generation sequencing, it is possible to obtain extensive genomic information on individual tumors in a very short time. Using this technology, it was reported that specific mutations might affect therapeutic efficacy and induce resistance to specific treatment.

Objective: The aim of this study was to investigate the mechanisms of taxane resistance using whole exon sequencing and expression analyses in human breast cancer tissues.

Materials and Methods: We selected six breast cancer patients whose tumors responded well to anthracycline treatment but suffered disease progression on taxane treatment. We then performed whole exon sequencing on these samples using HiSeq (Illumina). In this way, we identified somatic mutations of candidate genes considered to be instrumental for mediating resistance to taxanes. Next, we performed mRNA expression analyses of these candidate genes in a further 122 breast cancers treated with taxanes at our institute. Finally, we correlated mRNA expression levels of these genes with clinicopathological factors and prognosis.

Results: We identified 9 mutations common to all 6 patients analyzed in this study, and a further 16 mutations shared by 5 of them. Kaplan-Meier analyses showed that high level mRNA expression of 3 of these 25 genes was significantly associated with poorer disease-free survival. Moreover, high level mRNA expression of one of these three genes was significantly associated with worse overall survival. However, there were no significant correlations between expression levels of these three genes and any clinicopathological features.

Conclusion: Using next-generation sequencing, we have identified three candidate genes involved in resistance to taxane treatment in breast cancer. We are now analyzing the functional attributes of these three genes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-03-08

Title: Influence of mutagen exposure on molecular profile in breast cancer

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Body: Background: Molecular profiling of tumor DNA has changed the management of many cancers. For example, differences in mutations have been observed in lung adenocarcinoma between smokers and non-smokers, with the latter harboring more EGFR mutations. However, there is a paucity of information the influence of mutagen exposure on molecular profile in other cancers. This is a pilot analysis to determine if smoking history or prior adjuvant radiation has an influence on the molecular profiles of women with breast cancer.

Methods: Moffitt's Clinical Genomic Action Committee (CGAC) database was used to assess 62 patients with breast cancer treated at Moffitt with targeted deep sequencing of 315 genes as part of clinical care between 4/1/2013 and 12/31/2015. Smoking history was determined by self-report and history of adjuvant radiation therapy was abstracted from the medical record. Tumor sequencing results were analyzed for differential mutational burden (any alteration other than amplifications) and the presence of differentially altered genes between the groups of interest. The groups were similar with regards to percentage of triple negative patients (35.3% vs. 33.3%) between active/prior smokers and never smokers respectively.

Results: The most frequently mutated genes across the cohort were *TP53* (54.2%), *PIK3CA* (40.7%), *MLL3* (25.4%), *CDH1*, *PTEN*, and *MLL2* (18.6%), *BRCA2* and *NF1* (15.3%), *BARD1*, *ESR1*, *CDKN2A/B*, and *SPEN* (13.6%).

There was no difference in the mutational burden between 17 smokers (mean 10.2 alterations; range 1-21) and 42 never smokers (mean 11.5 alterations; range 4-30); $p=0.35$, with a trend towards more mutations in never smokers. Amongst genes with at least for total alterations, breast cancers of smokers contained a higher frequency of alterations in *APC*, *BLM*, and *RAD50* (17.6% vs. 2.4%), *PIK3CA* (58.8% vs. 33.3%), *MAP3K1* (23.5% vs. 7.1%), *GPR124* (17.6% vs. 4.8%), and *TP53* (70.6% vs. 47.6%); although none met statistical significance for multiple comparisons. On the other hand, non-smokers contained increased alterations in *MLL2* (5.9% vs. 23.8%), *ATM* (0.0% vs. 14.3%), as well as *FAT1*, *ATR*, *AR*, and *KDM5C* (0.0% vs. 11.9%); but also did not differ to a statistically significant degree.

There was no difference in the mutational burden between 26 patients without prior radiation and the 34 with prior radiation, each group had a mean of 11.1 alterations.

Conclusions: There was no difference in the mutational burden between smokers and never smokers in breast cancer or between breast cancer patients who received prior radiation therapy and those who did not.

Smoking Status	Mean # of Alterations	Range	Associated Molecular Alterations
Current/Former (n = 17)	10.2	1-21	APC, BLM, RAD50, PIK3CA, MAP3K1, GPR124, TP53
Never (n=42)	11.5	4-30	MLL2, ATM, FAT1, AR, KDM5C

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-03-09

Title: Comparison of the mutational landscape of breast cancer during pregnancy and non-pregnant controls

Loibl S, Pfarr N, Weber K, Neunhöffer T, Villegas S, Stenzinger A, Furlanetto J, Aktas B, Budczies J, Marmé F, Kahmann L, Denkert C and Weichert W. German Breast Group, Neu-Isenburg; Institute of Pathology, Technical University Munich; Helios Kliniken Wiesbaden, Wiesbaden; Institute of Pathology, Charité Berlin; Institute of Pathology, University Hospital Heidelberg; University Women's Hospital, Essen; NCT, Section Translational Gynaecologic Oncology, Heidelberg and Klinikum Landkreis Neumarkt.

Body: Background: Currently, breast cancer during pregnancy (BCP) is not believed to be biologically different from breast cancer unrelated to pregnancy based on limited datasets mainly obtained by immunohistochemistry. However, some groups report that BCP patients have an inferior survival compared to young non-pregnant breast cancer patients. The largest analysis based on the BCP registry by the German Breast Group (GBG) revealed however, no difference between pregnant and non-pregnant breast cancer patients, indicating that treatment rather than biology might be the reason for the inferior survival reported by others.

Methods: The BCP study (GBG 29/BIG 03-02) is a multicentre observational study for breast cancer during pregnancy. In tumour tissue collected within this study from pregnant M0 patients we investigated the following genes: *AKT1*, *ATM*, *BRAF*, *CBFB*, *CCND1*, *CDH1*, *CDKN2A*, *CTCF*, *EGFR*, *ERBB2*, *ESR1*, *FGFR2*, *GATA3*, *KRAS*, *MAP2K4*, *MAP3K1*, *MDM2*, *MED12*, *MYC*, *PIK3CA*, *PIK3R1*, *PTEN*, *RB1*, *RUNX1*, and *TP53* by massive parallel sequencing (MPS). This included patients with all molecular subtypes: HR+/-, HER2+/- . Sequencing was done on an IonTorrent Proton using a custom designed Breast Cancer Panel (BCPv2). This panel comprises 236 amplicons split into two primer pools and covers hotspot regions of 138 exons of the 25 genes.

To test the hypothesis that breast cancer diagnosed during pregnancy is biologically not different from breast cancer diagnosed in young non-pregnant women, we compared the molecular profiles obtained, with genetic data from M0 patients not known to be pregnant from TCGA with age \leq 45. TCGA data were pre-processed to be compatible to the targeted MPS datasets from pregnant patient.

Results: Material from 141 patients from the BCP study was available from which ultimately 109 fully evaluable MPS datasets could be obtained. In the TCGA data set 114 breast cancer patients \leq 45 years could be identified. Pregnant patients with breast cancer were significantly younger, had more often HR- tumours (59.6% vs 30.1%) but had less frequently grade 3 tumours (30.6% vs 48.2%). All other clinical variables showed no significant differences between pregnant and non-pregnant patients. In the BCP data, overall 106 mutations could be found. The most frequent mutations were detected in *TP53* (62%) and in *PIK3CA* (11.1%). In non-pregnant patients the mutation rates were different with 32.5% in *TP53* and 21.1% in *PIK3CA*.

Exact matching by variables age, HR, HER2 and grade yielded 40 patients from both datasets. In these subcohorts, still divergent mutational rates for *TP53* and *PIK3CA* between pregnant and non-pregnant women were noted, however, the differences failed to reach statistical significance.

Conclusions: Overall the mutational landscapes do not seem to be overtly different between pregnant patients and non-pregnant controls, although slight imbalances in mutational rates occurred, which might be partly explained by a selection bias and a small sample size after matching. Further comparisons using other datasets, looking into survival and regarding copy number variation are currently conducted.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P2-03-10

Title: A fit-for-purpose NGS system that reports *ERBB2* (*HER2*) mutations and copy number variants for clinical trials research and drug development

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Body: Introduction: HER2-positive breast cancers comprise 20-30% by subtype and represent the second poorest prognosis. In addition to HER2 signaling activation via protein overexpression or gene amplification (measured by IHC or ISH), somatic mutations in the *ERBB2* (*HER2*) gene have been identified in 2-4% of breast cancers and reported to drive HER2 pathway signaling and tumorigenesis. Irreversible HER2 tyrosine kinase inhibitors such as neratinib have been shown to potently inhibit breast tumors harboring somatic HER2 activating mutations and are under active clinical investigation. A targeted molecular characterization strategy is thus needed to support clinical trial research. Here, we present an integrated NGS system that identifies multiple classes of *ERBB2* aberrations, including single nucleotide variants, insertion/deletion mutations, and copy number variations (CNVs).

Methods: Genomic DNA was isolated from FFPE human tumor tissues or FFPE-embedded engineered cell lines expressing *ERBB2* mutations. Pre-analytical sample QC was performed by a novel qPCR assay that quantifies amplifiable DNA templates suitable for library preparation. NGS libraries were prepared using the QuantideX® NGS *ERBB2* Mutations assay, with two-pool PCR-based enrichment to detect multiple types of *ERBB2* genomic alterations. Libraries were sequenced (Illumina MiSeq®) and analyzed using QuantideX® Reporter software, directly incorporating the pre-analytical QC data from each DNA sample to improve variant call accuracy.

Results: The QuantideX® NGS *ERBB2* Mutations system targets 28% of *ERBB2* coding bases covering exons 8 and 17 - 24, including codons 713-989 of the tyrosine kinase domain. The assay also detects focal, whole-arm and polysomy-induced HER2 amplification using sentinel flanking amplicons, and amplicons covering CEN17 and copy number-neutral chromosomal regions identified in the TCGA breast cancer cohort. The assay was analytically validated in a CLIA-laboratory which included an analysis of 6 mixtures and titrations of synthesized DNA bearing 31 *ERBB2* mutations, 17 engineered cell lines and FFPE cell-line blocks, and 12 surgically-resected FFPE carcinomas. A multi-day/operator study revealed 100% sensitivity and PPV for detection of *ERBB2* SNVs, indels and complex variants down to 5% mutation positive in wild-type DNA. The NGS system was also used to profile *ERBB2* variants in 473 FFPE human tumor tissues, including 280 breast carcinomas. Mutation-positive samples were confirmed by an orthogonal NGS method. A subset of 46 FFPE tissue samples were assessed for CNVs by NGS and compared with matched droplet digital PCR (ddPCR) analysis. A correlation of $R^2 > 0.91$ between NGS and ddPCR underscores the potential of the assay to reveal both *ERBB2* mutations and copy number changes.

Conclusions: The QuantideX® NGS *ERBB2* system integrates optimized wet- and dry-bench elements to achieve broad and accurate detection of *ERBB2* mutations and copy number variants from challenging FFPE specimens. The technology has the potential to advance breast cancer clinical research and aid in the selection of patients for clinical trials, particularly those that assess HER2-directed therapies such as afatinib, lapatinib, and neratinib.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-03-11

Title: Genomic pattern of breast carcinomas carrying mutations of non-*BRCA* homologous recombination genes

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Body: Background

BRCA1 and *BRCA2* are involved in the homologous recombination (HR) double-strand DNA break repair and genomic patterns of breast tumors with defective *BRCA* are characterized by increased genomic instability. The pre-clinical and clinical studies show that tumors with defective *BRCA* or other HR genes can response to platinum and PARP inhibitors. However, the genomic pattern of tumors carrying mutations of non-*BRCA* HR genes are not investigated.

Methods

Genomic patterns of breast carcinomas were performed by comparative genomic hybridization (CGH) array containing 60000 probes covering the whole genome with an average spacing of 40kb. The frequency of gains and losses for each regions detected by probes was calculated by ratio thresholds of 0.25 and -0.25, respectively. Large-scale genomic structural aberration was defined as the region of gains and losses of at least 10Mb. We analyzed the difference of large-scale aberration, including numbers, length and specific regions, between tumors with *BRCA* mutation (mt*BRCA*), non-*BRCA* HR mutation (mtHR) and wild type.

Results

We examined 41 breast carcinomas, including 15 cases with *BRCA* mutations, 14 with non-*BRCA* HR gene mutations and 12 without mutations (control). The 14 non-*BRCA* HR gene were 1 *ATM*, 1 *BRIP1*, 1 *BARD1*, 1 *FANCA*, 2 *FANCB*, 1 *FANCI*, 1 *PALB2*, 2 *RAD50*, 2 *RAD51C* and 2 *RAD51D*. The number and length of large-scale genomic structural aberration of mt*BRCA* tumors were significantly higher than wild type tumors (number $p=0.005$; length $p=0.005$), indicating CGH can distinguish the mt*BRCA* from control tumors. We then checked the mtHR tumors, which also revealed significantly increased number and longer length of structural aberrations compared to wild type tumors (number $p=0.035$; length $p=0.022$), but were not different from mt*BRCA* tumors (number $p=0.204$; length $p=0.425$). Among the specific regions on chromosomes, mt*BRCA* and mtHR tumors contained similar genomic aberration regions but different from wild type tumors. The most frequent aberration regions of mt*BRCA* and mtHR tumors are chromosome 6p22.1-p25, 6q21-q27, 8q11.1-q24, 11p11.2-p14.1, 11q, 12p and 19p, which are less revealed in the wild type (all p value <0.05).

Conclusions

Our study demonstrated a direct evidence that increased genomic instability were the common characteristics of mt*BRCA* and non-*BRCA* mtHR tumors. In addition, we identify the specific genomic patterns of mt*BRCA* and mtHR tumors, which can be a biomarker indicating HR deficiency and response to platinum and PARP inhibitors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-01

Title: Increased stromal lymphocytes in breast cancer patients with autologous dendritic cell vaccination plus neoadjuvant chemotherapy

Baraibar I, Martin-Romano P, Mejías L, Perez Solans B, Cenicerros L, Legazpi J, Gardezabal I, Inoges S, Lopez-diaz A, Idoate M, Cordoba A and Santisteban M. Clinica Universidad de Navarra and Hospital Virgen del Camino.

Body: Introduction: Higher levels of TILs after neoadjuvant chemotherapy (NAC) in the residual tumor have been related to a better outcome in BC patients. According to International TILs working group (2014), we have quantified stromal TILs in a cohort of patients treated with NAC plus dendritic cell vaccines (cohort V) (NCT01431196 plus an expanded cohort) and in an historic cohort of patients treated with the same NAC without V (cohort C) in order to assess if the addition autologous dendritic cell vaccination pulsed with patient's tumor lysates helps to increase TILs in the residual BC specimen. Our previous data with these cohorts of patients have shown an improved pCR and the stimulation of lymphocytes population in the peripheral blood from cohort V.

Methods: Classification of molecular subtypes was performed by IHC. We evaluated percentage of TILs by three different pathologists using stained HE core biopsy sections in paired samples taken at diagnosis (preNAC) and after surgery (postNAC) in a prospectively defined retrospective analysis.

Results: 79 patients were evaluated (cohort C 42; cohort V 37). Patients from V cohort were younger ($p=0.01$). Cohorts were well balanced for AJCC staging, molecular subtypes (cohort C: LA 26%, LB 45%, TN 29%; cohort V: LA 24%, LB 40%, TN 35%), stromal TILs preNAC [cohort C: median 9 (range, 1-24); cohort V: 6 (1-34); $p=0.38$] and stromal TILs postNAC [cohort C: 9 (2-52); cohort V 8 (4-50); $p=0.94$]. Seventy-seven patients (97%) underwent surgery (cohort C 41; cohort V 36). Pathologic CR was higher in Cohort V (27% vs 7%; $p=0.02$). However a superior pCR rate was detected in TNBC subtype (cohort C 27%; cohort V 58%; $p=0.15$). Patients from Cohort V experienced an increment of stromal TILs after NAC plus autologous dendritic cell vaccination (preNAC: 6 (1-34); postNAC 8 (4-50); $p=0.03$), especially in TNBC [preNAC 14 (1-34); postNAC 30 (10-50); $p=0.2$]. A correlation analysis by Pearson's R coefficient stated a strong association in V cohort between stromal TILs preNAC and postNAC ($R=0.83$) as well as a moderate correlation between stromal TILs postNAC and molecular subtypes ($R=0.6$).

Table 1. Differences in Stromal TILs between studied cohorts

	PreNAC (median, range)	PostNAC (median, range)	P value
Cohort C	9 (1-66)	9 (2-52)	0.22
Cohort V	6 (1-34)	8 (4-50)	0.03*
Cohort C LA LB TN	5 (1-10) 9 (1-24) 12 (10-24)	6 (3-12) 10 (2-40) 13 (5-52)	0.69 0.17 1
Cohort V LA LB TN	5 (2-17) 6 (1-15) 14 (1-34)	7 (4-12) 7 (4-27) 30 (10-50)	0.18 0.08 0.2

Conclusion: Pathological complete responses were higher in BC patients treated with neoadjuvant chemotherapy plus active immunotherapy. The addition of autologous dendritic cell vaccines to NAC increases significantly stromal TILs in breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-02

Title: Recognition of autologous neoantigens by tumor infiltrating lymphocytes derived from breast cancer metastases

Goff SL L, Zacharakis N, Assadipour Y, Prickett T, Gartner J, Somerville R, Black MA A, Xu H, Chinnasamy H, Kriley I, Lu L, Statler M, Wunderlich J, Robbins PF F, Rosenberg SA A and Feldman SA A. NIH/NCI Surgery Branch, Bethesda, MD.

Body: Background & Translational Relevance: Adoptive transfer of tumor infiltrating lymphocytes (TIL) can effect long-term durable regression in patients with metastatic melanoma but has not been widely tested in common epithelial cancers. When examining the TIL of successfully treated patients with melanoma, a heterogeneous T cell population can be identified with reactivity against melanoma differentiation antigens, cancer germline antigens, and personalized non-synonymous somatic mutations. Common epithelial cancers, including breast cancer, express far fewer somatic mutations than melanoma, however, a patient with metastatic cholangiocarcinoma was treated with autologous CD4+ TIL enriched for neoantigen specificity and has experienced an ongoing partial response (>2 years). It is known that the presence of TIL on pathologic examination of triple-negative breast cancers is a positive prognostic marker for disease-free survival and overall survival. The identification of enriched populations of neoantigen-specific TIL could form the basis for personalized cell therapy for patients with metastatic breast cancer. This pilot study investigates the ability to grow TIL from breast cancer metastases, to identify personalized non-synonymous somatic mutations and potential neoantigens, and to adoptively transfer TIL into patients with breast cancer.

Methods: Eligible patients were evaluated and treated under IRB-approved protocols for tissue procurement, genomic testing, and adoptive cell transfer. Portions of resected tumors were placed in culture under standard TIL conditions. DNA was extracted from tumor and matched normal peripheral blood samples for whole exome sequencing (WES). Non-synonymous somatic mutations were identified and tested for potential immunogenicity.

Results: Nine patients have undergone surgical resection in this ongoing pilot study, and TIL was successfully grown from the tumors of all patients. All were primarily CD3+ (median 79%) with a small population of natural killer cells. Of the CD3+ cells, 7 of 9 patients had a predominantly CD4+ population (median CD4:CD8 ratio 2.2, range 0.4-5.8). With the exception of a single patient with inflammatory breast cancer, tumor purity allowed for WES of the tumors of eight patients, and non-synonymous somatic mutations were identified as potential neoantigens (median 96.5, range 71-148). Autologous T cell reactivity has been identified against tumor-specific mutations in 4 of 6 patients studied. The TIL of one patient demonstrated *in vitro* reactivity to a mutated form of RBPJ, a DNA-binding protein involved in Notch1 signaling. In addition, specimens obtained from this patient at autopsy contained the specific RBPJ mutation (RBPJ c.A611T) in every sampled tumor (n=16). Other patient-specific neoantigens identified by autologous reactivity include SLC3A2, KIAA0368, and a mutated TCRBV domain.

Conclusions: Tumor-infiltrating lymphocytes derived from metastatic breast cancer can react to tumor-specific non-synonymous somatic mutations *in vitro*. TIL grown from breast cancers are predominantly CD4+ and can form the basis of an adoptive cell transfer experimental approach to patients with metastatic breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-03

Title: Differential effect of chemotherapy on immune gene expression signatures based on molecular subtype of breast cancer

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Body: Background: Neoadjuvant chemotherapy (NAC) is frequently used in triple negative breast cancers (TNBC), and patients who achieve pathological complete response (pCR) following NAC have improved outcomes over those with residual disease (non-pCR). Unfortunately only 30% of TNBC patients achieve pCR, with no good treatment options for other 70% with non-pCR. Tumor infiltrating lymphocytes (TILs) in TNBC are predictive of pCR to NAC, and TILs found in the residual disease further prognosticate patients with residual disease into an improved prognosis subset. These data suggest there is an immune component to TNBC that might be affected by chemotherapy. Given the advent of immunotherapy trials in breast cancer, specifically in combination with NAC, there is an unmet need to gain a better understanding of the immune microenvironment in TNBC.

Objective: We investigated the role of NAC on the immune microenvironment by examining breast cancer samples before (diagnostic biopsy) and after (residual disease) NAC, as these data are pertinent to the design and analysis of ongoing clinical trials in breast cancer combining immunotherapy with concurrent chemotherapy.

Methods: RNA was extracted from 51 paired (pre- and post-NAC) breast cancer specimens with extensive residual disease, and analyzed by nCounter analysis for expression of >750 immune-related genes. Functional immune signature scores were generated, compared between matched pre- and post-NAC, and stratified by breast cancer molecular subtype (luminal (n=4), Her2-enriched (n=5), basal-like (n=17)). DNA extracted from a subset of these samples was utilized for T cell receptor sequencing to explore changes in T cell clonality following NAC in each subtype.

Results: Across all subtypes of breast cancer, immune scores decreased after NAC consistent with a broad decrease in TILs observed histologically. When samples were stratified by molecular subtype using the PAM50 analysis, luminal A/B and basal-like patients demonstrated a decrease in immune signatures after NAC, while Her2-enriched patients exhibited a global increase in immune scores. Importantly, basal-like patients had the greatest immune signature changes, including decreases in T cell functions and CD8+ T cell, T helper and T regulatory signatures ($p > 0.05$). Conversely, these signatures were increased after NAC in the Her2-enriched molecular subtype. Analyses of T-cell clonality are ongoing, but should yield insight into the effect of NAC on the landscape of effector T-cells in the micro-environment.

Conclusions: Our work suggests that NAC decreases immune infiltrate signatures, specifically related to effector T cells in patients who do not achieve pCR. However, we observed an increase after NAC in the same signatures in Her2-enriched patients, suggesting that these patients' tumors may respond differently to chemotherapy on the immune-molecular level. As clinical trials progress in TNBC with the combination of chemotherapy and immune therapy, more work is needed to understand who might benefit from these therapy regimens.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-04

Title: *BRCA* gene mutations do not shape the extent and organization of tumor infiltrating lymphocytes in triple negative breast cancer

Willard-Gallo K, Solinas C, Marcoux D, t'Kint de Roodenbeke D, Garaud S, Van den Eynden G, de Wind A, Boisson A, Larsimont D and Piccart M. Institut Jules Bordet, Universite Libre de Bruxelles, Brussels, Belgium and GZA Ziekenhuizen, Wilrijk, Belgium.

Body: The remarkable responses observed in metastatic cancer patients treated with immunotherapies, including inhibitors directed to the PD-1 and PD-L1 checkpoint molecules, makes it a priority to identify critical variations in pro- and anti-tumor immune responses in breast cancer (BC). In patients with triple negative (TN) BC, an increased presence of tumor infiltrating lymphocytes (TIL) and tertiary lymphoid structures (TLS) have been associated with good clinical outcomes. However, the frequency of specific lymphocyte subpopulations, PD-1 and/or PD-L1 expression and their prognostic significance remains an open question. Our recent work found that PD-1 and PD-L1 expression are specifically associated with higher TIL densities and an increased number of TLS in BC. We further demonstrated that TIL density, TLS and PD-L1 expression were correlated with more aggressive breast tumor characteristics, including higher proliferation and hormone receptor negativity. In this project, we examined the prevalence of TIL, TLS, PD-1 and PD-L1 expression in TNBC and further compared these immune parameters between TNBC patients harboring *BRCA1* or *BRCA2* germline gene mutations with those carrying the wild-type (wt) genes. A total of 1402 BC patients whose blood was genetically tested for germline *BRCA1* and *BRCA2* mutations were examined for inclusion in this study. Ninety-eight chemotherapy-naïve patients with primary invasive ER⁻, PR⁻ and HER2⁻ BC and demonstrated germline *BRCA1* or *BRCA2* wt or mutated-gene status were included in this study. Ninety-four tumors were determined to be suitable for evaluating immune cell infiltration (51 *BRCA* wt and 43 *BRCA*-mutated). FFPE tumor tissue from the surgical specimens was analyzed by immunohistochemistry (IHC) staining of full-face tissue sections. IHC was performed as a dual label using CD3 plus CD20 for T and B cells, CD4 plus CD8 for the major T cell subpopulations and PD-1 plus PD-L1 for individual or paired expression of these receptors. The stained slides were independently scored by two experienced pathologists for TIL, TIL subpopulations, TLS and checkpoint molecule expression.

These analyses revealed that 87% of our TNBC cohort was TIL-positive ($\geq 10\%$ TIL) with 35% classified as lymphocyte predominant BC (LPBC; $\geq 50\%$ TIL). T cells were the principal component of the lymphocytic infiltrate with no significant differences between the *BRCA* wt and *BRCA*-mutated groups detected in total T cells (CD3⁺), helper T cells (CD4⁺), cytotoxic T cells (CD8⁺) or B cells (CD20⁺). TLS were identified in 73% of tumors with again no significant differences between the *BRCA* groups. Examination of checkpoint molecule expression identified 33% tumors as PD-1 positive and 40% as PD-L1 positive. PD-1 expression was correlated with PD-L1 expression and both with TIL positivity and the level of immune infiltration but not *BRCA* mutational status.

Overall, our analyses revealed that *BRCA* wt and *BRCA*-mutated TNBC are remarkably similar in terms of TIL heterogeneity, a TLS presence and checkpoint molecule expression. These data suggest that *BRCA* gene mutations are not immunogenic nor do they directly drive immune infiltration in TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-05

Title: Immunomodulation effects of metronomic oral Vinorelbine (mVRL), with or without capecitabine (CAPE), on Treg levels in advanced breast cancer (ABC) patients (pts). Preliminary results of the VICTOR-5 study

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Body: BACKGROUND

In cancer patients, the accumulation of Tregs is associated with tumor progression and the suppression of anti-tumor immune response. Metronomic cyclophosphamide (mCTX) induces a profound and selective reduction of circulating Tregs, whereas no data are available regarding a possible effect on immune system. In the present analysis, we report preliminary data of Treg frequencies and function during a period of treatment of 2 months and correlations with anti-tumour T-cell response, in a group of HR+/HER2ve ABC pts.

PATIENTS AND METHODS

Following approval by the Ethical Committee, a sample of 3 ml of peripheral blood was drawn from 12 ABC pts for which mVRL 40- 50 mg thrice a week (N=10), \pm mCAPE 1500 mg/day (N=2), was indicated. Median age was 66,5 years (45-86); 2/ 12 received the mCHT as 1st-line therapy, 10/12 as 2nd-line or further. Blood samples were collected at baseline (T0) and after 14 (T1), 28 (T2), 42 (T3) and 56 days (T4) of treatment. Total lymphocytes (TL) and lymphocyte subgroups were determined according to Naack et Al guide lines. The Treg subpopulations have been identified by monoclonal antibodies CD45 V500, CD3 V450, CD4 PerCP-Cy5.5, CD25 PE, CCR4 PE-Cy7, CD 27 Alexa 647, CD45RO APC-H7, CD28 FITC, (BD Biosciences, San Jose, CA) and analyzed with BD FACS Canto™ II (Becton Dickinson, San Jose, CA), technic Lyse/Wash and software FACSDiva™.

RESULTS

Data for the purpose of this analysis are available for 10 out of 12 enrolled pts. mVRL \pm mCAPE induced a significant reduction of circulating Treg in 6/10 pts (60%) – Group A - at day 14. Median percentages of Treg among CD4+ cells were 9.4% \pm 1.5% SE at baseline vs 6.8% \pm 4.5% SE and 7.6% \pm 1.2% SE after 14 and 28 days of treatment in Group A. In patients without Treg depletion – Group B – median percentages of Treg were 8.4% \pm 0.9% SE, 9.6% \pm 1.3%SE and 8.2% \pm 1.5%SE as measured at the same time points. The depletion of Treg is associated with a slight expansion of CD8+ cells in Group A at all times of evaluation. No increase in CD8+ population has been observed in Group B. Median percentages of Treg and CD8+ cells in the two Groups are reported in Tables 1 & 2.

Frequency of CD4+CD25+ (Treg) among total CD4+ T cells in Group A (pts with Treg depletion) and Group B (pts w/o Treg depletion)

Time of evaluation	Mean Treg % +/- SE% - Group A	Mean Treg % +/- SE% - Group B
T0 (baseline)	9.4% +/- 1.5%	8.4% +/- 0.9%
T1 (+14 days)	6.8% +/- 4.5%	9.6% +/- 1.3%
T2 (+28 days)	7.6% +/- 1.2%	8.2% +/- 1.5%

Frequency of CD8+ cells among total lymphocytes in Group A (pts with Treg depletion) and Group B (pts w/o Treg depletion)

	Mean CD8+ (%) +/- SE% - Group A	Mean CD8+ +/- SE% - Group B
T0 (baseline)	20.6% +/- 1.9%	30.6% +/- 3.8%

T1 (+14 days)	21.6% +/- 1.9%	30.6% +/- 4.3%
T2 (+28 days)	225.5 +/- 1.9%	30.8% +/- 4.7%

CONCLUSION

Our results suggest that mVRL induces different immunomodulation effects in an unselected population of ABC pts. Treg depletion seems to increase the adaptive immune response. Data obtained from a longer follow up will be presented. These findings are hypothesis-generating for future evaluation of mVRL as a priming agent to increase response to anti PDL-1 agents.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-06

Title: Targeting of the unfolded protein response signaling arms differentially regulates macrophage proliferation, plasticity, and breast cancer cell clearance

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Body: The unfolded protein response (UPR) is an endoplasmic reticulum stress pathway controlled by the protein chaperone, glucose-regulated protein 78 (GRP78), to mediate inositol-requiring enzyme 1 (IRE1), PKR-like endoplasmic reticulum kinase (PERK), and activating transcription factor-6 (ATF6) signaling. UPR signaling has been shown to be upregulated in many different types of cancers, including breast cancer and melanoma, and is associated with the development of therapeutic resistance. These data suggest the importance of targeting UPR signaling as a possible cancer therapy. We have previously shown GRP78 to be upregulated in human breast tumor samples and leads to endocrine targeted therapy resistance. We recently showed inhibiting GRP78 in human orthotopic xenografts potentiates tamoxifen therapy effectiveness in sensitive tumors and restores endocrine therapy responsiveness in resistant tumors. In these GRP78-inhibited tumors there was a significant increase of CD68 positive macrophage population, suggesting that targeting UPR signaling has critical effects on the tumor microenvironment. Therefore, consideration of each UPR signaling component and how it effects the different cellular compartments of the tumor microenvironment need to be investigated to optimally induce both an antitumor immune effect and inhibit tumor epithelial cell growth. We now show deletion of GRP78, IRE1, and PERK through RNAi differentially regulates macrophage polarization. Specifically, PERK inhibition enhances macrophage proliferation and macrophage-mediated phagocytosis of 4T1 breast cancer cells, but not GRP78 or IRE1 inhibition. Targeting UPR signaling in the breast cancer cells also differentially affected macrophage cytolytic capacity; Specific breast cancer cell inhibition of IRE1 or GRP78 enhanced macrophage-mediated phagocytosis. Conditioned media from control or GRP78 silenced ZR-75-1 breast cancer cells indicated reciprocal regulation of CD206 and CD80; suggesting regulation of macrophage plasticity by GRP78-controlled secreted factors. GRP78 targeting in mice resulting in a cytokine shift and increased tumoral CD80+/CD68+ cells, suggesting that GRP78 inhibition favors a M1-like macrophage profile. Inhibition of UPR components in both macrophage and breast cancer cells, similar to what would be observed in systemic cancer therapies, indicated that either PERK or GRP78 inhibition enhances macrophage cytolytic clearance of breast cancer cells. Taken together, these data suggest that targeting GRP78 or PERK promotes an anti-tumor immune response by either directly promoting macrophage cytolytic activity (PERK targeting) or indirectly by shifting tumoral cytokine secretion (GRP78 targeting).

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-07

Title: Immune profiling of post neoadjuvant high metastatic risk (RCB-II/III) residual disease in patients with early triple negative breast cancers

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Body: Background: Poor prognosis in TNBC can be predicted in the significant fraction of patients with large volume residual cancer burden (RCB-II/III) after neoadjuvant chemotherapy (NACT). Whilst residual disease has been characterised to identify “driver” mutations and copy number variations, the contribution of the immune response within its tumour microenvironment remains unclear. Here we aimed to: 1) assess the potential spatial heterogeneity of immune transcript related gene expression between areas of tumour approximately 1cm apart as might still occur with a radiologically guided biopsy through the residual disease; and 2) assess the immune stroma composition of the TNBC high metastatic risk RCB II/III disease.

Method: 12 TNBC post NACT RCB II/III residual cases were identified from the KHP biobank. H&E sections were reviewed and areas of tumor 1cm apart within a residual resection specimen marked as area A and area B. HistoQuest analysis software was used to quantify the proportion of tumor infiltrating lymphocytes (per total cell count) within both areas. RNA was extracted from both areas and immune gene expression profiling performed using a Nanostring nCounter® on all 24 samples. The immune PanCancer panel consisted of 770 genes combining markers for different immune cell populations. Differential genes between paired samples were compared and unsupervised hierarchical clustering using 770 genes and immune cell types performed.

Results: Quantitative comparison of the tumour infiltrating lymphocytes (TILS) between area A and B revealed that 73% (8/11) of the cases had a <2-fold difference in the percentage of TILS within a residual specimen; and 27% (3/11) displayed a >2-fold (range 2.03-3.16) difference in the TILS. When comparing the 770 gene expression profiles between sampling areas in the same tumour, we found little spatial heterogeneity with areas A/B clustering together in 10 out of 12 cases. Interestingly, the two cases that revealed spatial heterogeneity within the paired samples displayed little immune cell heterogeneity histologically (i.e. <1-fold change in the TILS percentage score between area A and B). Comparing patient samples by immune gene expression profiling divided the patients into two groups: i) those with immunologically enriched tumors in whom gene signatures for majority of the immune cell types (DC, Macrophages, CD8+T-cells, T-helper cells (Th17, Th2, Th1), Tregs, NK, B-cells, Neutrophils, Mast cells) were highly expressed and ii) those with immunologically inert tumors in whom the immune cell signatures were not highly expressed. Within our patient cohort, patients with immunologically enriched gene expression profiles were also seen to display higher TILS score (ranging between 28.17% to 40.66%) as compared to patients with immunologically inert gene expression with scores ranging from 11.82% to 16.80%.

Conclusion: The findings that high metastatic risk residual disease can be further characterized as either “immunologically inert” or “immunologically enriched” at the level of extensive immunological transcript gene expression and by histological assessment of TILS requires further investigation; and is being validated in a larger sample set.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-08

Title: BGB324, a selective small molecule inhibitor of the receptor tyrosine kinase AXL, enhances immune checkpoint inhibitor efficacy in mammary adenocarcinoma

Lorens JB B, Lipinska KW Wnuk, Davidsen K, Blø M, Hodneland L, Engelsen A, Kang J, Lie MK Kolnes, Bougnaud S, Aguilera K, Ahmed L, Rybicka A, Nævdal EM Milde, Deyna P, Boniecka A, Straume O, Chouaib S, Brekken RA A and Gausdal G. BerGenBio AS, Bergen, Norway; Biomedicine, Bergen, Norway; Center for Cancer Biomarkers, University of Bergen, Bergen, Norway; Haukeland University Hospital, Bergen, Norway; INSERM Unité 1186, Institut Gustave Roussy, Université Paris-Sud, Villejuif, Paris, France and Hamon Center for Therapeutic Oncology Research, UT Southwestern Medical Center, Dallas, TX.

Body: The AXL receptor tyrosine kinase is associated with poor overall survival in breast cancer. Axl signaling is an important regulator of tumor plasticity related to epithelial-to-mesenchymal transition (EMT) and stem cell traits that drive metastasis and drug resistance. Signaling via AXL is also a key suppressor of the anti-tumor innate immune response. AXL is expressed on several cells associated with the tumor immune microenvironment including natural killer cells, dendritic cells and tumor-associated macrophages. AXL is required for tumor immune evasion in mammary adenocarcinoma models and EMT-mediated resistance to cytotoxic T cell and natural killer (NK)-cell mediated cell killing. Hence AXL signaling contributes uniquely to both tumor cell intrinsic and microenvironmental anti-tumor immune suppression mechanisms in breast cancer. We evaluated whether blocking AXL signaling with BGB324, a selective clinical-stage small molecule Axl kinase inhibitor, enhances the effect of immune checkpoint blockade in the aggressive mammary adenocarcinoma (4T1) syngeneic (Balb/C) mouse model that display limited immunogenicity.

Immune therapy with anti-CTLA-4/anti-PD-1 increased AXL and EMT-marker expression in 4T1 tumors, and correlated with lack of response to immune therapy. Combination treatment with BGB324 (50 mg/kg bid) significantly enhanced responsiveness to anti-CTLA-4/anti-PD-1 treatment (10 mg/kg of each, 4 doses) in Balb/C mice bearing established 4T1 tumors. The combination of BGB324 + anti-CTLA-4/anti-PD-1 resulted in durable primary tumor clearance in 23 % of treated mice versus 5.6% obtained with anti-CTLA-4/anti-PD-1 alone ($p=0.0157$). In a separate study, BGB324 + anti-CTLA-4 treated resulted in 22% long-term primary tumor clearance while no response was observed with anti-CTLA4 treatment alone. The extensive metastasis to the lung, liver and spleen characteristic of this model were concomitantly abrogated in the animals responding to the combination treatment. In addition, BGB324 + anti-CTLA-4/anti-PD-1 treated tumors displayed enhanced infiltration of cytotoxic T lymphocytes (CTLs). Enhanced presence of CTLs was also detected in spleens from animals responding to treatment. BGB324 + anti-CTLA-4/anti-PD-1 treatment increased the number of NK cells, macrophages and polymorphonuclear neutrophils, but decreased the number of mMDSC. Importantly, responding animals rejected orthotopic 4T1 tumor cell re-challenge, demonstrating sustained tumor immunity.

Together with recent results in other tumor types that support a prominent role for AXL in resistance to immune therapy and encouraging results from ongoing clinical trials with BGB324, support combining BGB324 with immune checkpoint inhibitors to improve treatment of breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-09

Title: The prognostic and predictive effect of tumor infiltrating lymphocytes is not determined by B-cells or PD-L1 expression in inflammatory breast cancer

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Body: Background

The tumor stroma with tumor infiltrating lymphocytes (TILs) plays a crucial role in the aggressive inflammatory breast cancer (IBC) phenotype and a gene signature enriched for immunity-related genes showed that response to neo-adjuvant chemotherapy was associated with immunity related processes in IBC. In both IBC and non-inflammatory breast cancer (nIBC), tumors are infiltrated by B-cells, but their role in regulating anti-tumor immunity is not well understood. In this study we looked at the prognostic and predictive effect of B-cells in the immune infiltrate of 178 IBC and 247 nIBC patients.

Methods

TIL scoring was done on standard H&E stained sections of formalin-fixed paraffin-embedded pre-treatment tumor tissue according to international guidelines (Salgado et al., 2015). B-cells in the immune infiltrate were defined as CD79 α (clone JCB117) positive cells and scoring was done semi-quantitatively, both intra- and peritumorally. Slides, stained with a validated PD-L1 assay (clone SP142) were scored on immune cells (IC) according to Herbst et al., 2014.

Results

Most of our IBC patients presented with a grade 3 (67.7%), ductal (91.1%) carcinoma. A quarter of the patients (25.7%) with initially localized disease (71.6%) achieved complete pathological response (pCR) after neo-adjuvant chemotherapy. The mean TIL score was 18.02% (1.0 – 80.0) and 67 out of 156 patients (42,9%) were PD-L1 positive. Categorical scores of the immune infiltrate are summarized in the table.

	Category 1 (< 10%)	Category 2 (\geq 10, < 40%)	Category 3 (\geq 40 %)
TIL score (n=178)	34.73% (n= 61)	53.4% (n= 95)	12.4% (n=22)
Intratumoral CD79 α score (n= 175)	69.1% (n= 121)	22.3% (n= 175)	8.6 % (n= 15)
Peritumoral CD79 α score (n= 171)	48.5 % (n= 83)	38.0 % (n= 65)	13.5 % (n= 23)

TILs were significantly higher in oestrogen receptor negative (ER-) groups compared to ER+ groups (21.26% vs. 15.58%, p= 0.017). In IBC, univariate analysis showed that achieving pCR was significantly associated with more TIL infiltration (p< 0.001), PD-L1 IC expression (p= 0.013) and intratumoral CD79 α scores (p= 0.036). However, in multivariate analysis the effect of PD-L1 and CD79 α positive cells was lost. When corrected for different molecular subtypes the predictive effect of TILs was only present in ER- groups. Survival analysis showed a significant beneficial effect of TILs (p= 0.014) but only in the ER+ groups, while PD-L1, intra- and peritumoral CD79 α scores were not significant. Intratumoral CD79 α scores correlated with both TIL score (p= 0.024) and PD-L1 expression (p= 0.007) in multivariate analysis.

When comparing IBC to nIBC, TILs were significantly higher in IBC patients (15.58 % vs. 11.29%, p= 0.009) in the ER+ group, while in the ER- group they were significantly lower (21.27% vs. 37.19%, p < 0.001). This was also seen for CD79 α peritumoral positivity, but logistic regression revealed that this was a TILs effect.

Conclusion

A high TIL score correlates with a better OS and pCR in IBC, depending on the ER status. B-cells and PD-L1 expressing IC

appear not to contribute to this effect, but do correlate independently with each other. Further research is needed to unravel the driving and targetable components of the immune responses in IBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-10

Title: High-affinity activated natural killer (haNK) cells augment trastuzumab efficacy in a mouse model of HER2-positive human metastatic breast cancer

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Body: Background. The ability of NK cells to kill cancer cells makes them an attractive choice for clinical immunotherapy. Early phase clinical trials in patients (pts) with advanced cancers have demonstrated the safety of activated NK (aNK [NK-92]) cells, an investigational cell line that was established from the peripheral blood mononuclear cells of a pt with non-Hodgkin lymphoma. NK cells can participate in antibody-dependent cellular cytotoxicity (ADCC) mediated by recognition of the Fc fragment of the target-bound antibody (IgG₁) via the CD16 Fc receptor. Among pts with HER2-positive breast cancer treated with trastuzumab (IgG₁) the high-affinity CD16 V/V genotype was significantly correlated with better clinical outcomes (Musolino. *JCO*. 2008;26:1789-96). To enhance the killing activity of aNK cells, we modified aNK cells to stably express high-affinity CD16 and evaluated the resulting haNK cells in combination with trastuzumab in a mouse xenograft model of HER2-positive human breast cancer.

Methods. haNK cells were generated by transfection of aNK cells with a bicistronic plasmid coding for CD16 (158V) and an intracellular form of IL-2, which enables haNK cells to grow in the absence of exogenous IL-2. Female, 7 to 8-week-old NOD-scid IL2Rgamma^{null} (NSG) mice were inoculated subcutaneously in the left and right flank area with 0.1mL of 1x10⁸/mL MDA-MB-453 human breast cancer cells in 50% Matrigel. When tumors reached ≥100mm³, mice were randomly assigned to 10 groups of 4 mice per group and dosed (IV) with PBS, 1 or 3mg/kg IgG₁ isotype control, 1 or 3mg/kg trastuzumab (determined from a dose range finding study), 1x10⁷ non-irradiated haNK cells, or non-irradiated haNK cells in combination with IgG₁ or trastuzumab. The dosing schedules were: PBS/haNK cells twice weekly for 4 weeks; IgG₁/trastuzumab once weekly for 4 weeks. For the combination treatments, mice received antibodies at least 3h prior to the injection of haNK cells. Tumor growth and animal weights were measured twice weekly.

Results: Results obtained after 4 weeks of treatment are shown in the table. haNK alone and both doses of trastuzumab alone significantly inhibited the growth of human MDA-MB-453 breast tumors. The combination of haNK plus 1mg/kg trastuzumab was synergistic with a T/C value of -60.1%.

Conclusions: The combination of haNK cells and trastuzumab was synergistic at 1mg/kg trastuzumab resulting in tumor regressions and significantly better efficacy vs each agent alone. Trastuzumab monotherapy at 3mg/kg was very effective and likely masked any synergistic effect of haNK. This study illustrates the potential for combining haNK cells with trastuzumab in a clinical trial of pts with HER2-positive metastatic breast cancer.

Results

Group	Treatment	T/C (%)	BWC (%)
A	PBS	-	-0.6
B	IgG ₁ (1mg/kg)	40.8	-4.7
C	Trastuzumab (1mg/kg)	-34.5	-2.8
D	haNK (1x10 ⁷)	-20.3	-15.9
E	IgG ₁ (1mg/kg) + haNK (1x10 ⁷)	-10.7	-16.0
F	Trastuzumab (1mg/kg) + haNK (1x10 ⁷)	-60.1	-16.6
G	IgG ₁ (3mg/kg)	39.1	-0.4
H	Trastuzumab (3mg/kg)	-95.2	-1.8
I	IgG ₁ (3mg/kg)+ haNK (1x10 ⁷)	-26.4	-16.7
J	Trastuzumab (3mg/kg) + haNK (1x10 ⁷)	-93.8	-18.3

%T/C, percent treated/control; BWC, body weight change.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-11

Title: Promotion of immunogenicity using epigenetic modulation and immune checkpoint inhibition in mouse models of breast cancer

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Body: Checkpoint inhibition has been a very successful treatment strategy in cancers that are naturally immunogenic by attracting T cells into the tumor microenvironment (TME) and promoting cytotoxic signaling pathways. While this strategy has shown some efficacy in metastatic breast cancer, most breast cancers are not highly immunogenic likely due to an immunosuppressive microenvironment and a lack of tumor antigen expression and recognition. One strategy to transform the breast TME into one that is immune responsive, is to use epigenetic modulation to expose tumor antigens and promote cytokine production, which in turn will attract T cells into the tumor. Additionally, dissection of the specific inhibitory mechanisms including checkpoint pathways that inhibit T cell attraction and function in breast tumors, will inform combination therapies that effectively activate the immune response. This approach could open the door for successful immunotherapy approaches for breast cancer patients. We are using two different mouse models, a syngeneic transplantable model using the triple negative breast cancer cell line 4T1 in BALB/C mice; as well as the Her-2/neu (neu-N) transgenic model. These models will allow us to study the efficacy of different combinations of an epigenetic agent, the histone deacetylase inhibitor Entinostat, and checkpoint inhibitors anti-programmed cell death protein (PD1) and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) antibodies, on tumor growth and metastatic progression, and to help identify co-stimulatory and inhibitory factors regulating T cell responses. Characterization of tumor infiltrating lymphocytes and their functional capabilities are being investigated in both primary tumors and within metastatic foci using fluorescence-activated cell sorting and immunohistochemistry. BALB/c mice were inoculated in the mammary fat pad with the highly metastatic 4T1 cell line. Thus far, there seems to be a trend toward incomplete tumor regression in mice treated with combination anti-PD1, anti-CTLA4 and Entinostat. There is also a trend toward improved survival in the combination treatment group. Additionally, similar results are seen using the transgenic neu-N mouse model whereby, combination therapy leads to a delay in tumor progression and improved survival. Preliminary results also suggest that epigenetic modulation with Entinostat improves the immune response within these tumors as evidenced by detection of different T-cell populations and myeloid derived suppressor cells (MDSCs) within treated tumors. Thus far, in both the 4T1 and neu-N models of breast cancer, treatment with Entinostat seems to prime the TME to be more immunogenic by altering the population of T cells and MDSCs in the tumor. Combination therapy with anti-PD1 and anti-CTLA4 affect tumor growth and further modulate the immune response; however, the specific signaling pathways involved have yet to be elucidated. Given these observations, we hypothesize that triple combination therapy will significantly affect specific immune modulatory pathways and we hope to uncover the specifics of how these pathways are leading to improved overall outcome.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-12

Title: Targeting of phosphatidylserine by monoclonal antibodies enhances the activity of immune checkpoint lag3 targeting antibodies in murine breast tumors

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Body: Previous preclinical modeling has demonstrated that antibodies targeting the programmed cell death ligand 1 (PD-1) have activity in murine breast cancers (BC), and this activity is significantly enhanced when combined with phosphatidylserine (PS) targeting antibodies. This suggested that the addition of PS targeting antibodies could be capable of augmenting anti-tumorigenic properties of other checkpoint inhibitors and alternative immune activating therapies in BC. The ability of PS targeting antibodies to enhance the activity of anti-PD-1 therapy occurs in part through increasing tumor infiltrating lymphocytes (TILs), boosting the Th1 immuno-profile, and the suppression of tumor promoting cytokines induced by anti-PD-1 therapy. PS normally resides in the inner leaflet of the plasma membrane in many types of cells. Conditions that incite cellular stress in the tumor microenvironment, such as ROS, hypoxia, and irradiation, promote PS externalization and exposure on tumor associated endothelial cells and tumor cells, where it is recognized by specific receptors, including members of TIM and TAM family. This PS recognition promotes an innate system driven immunosuppressive condition in part by promoting the recruitment of myeloid derived suppressor cells (MDSCs), M2-like macrophages, and suppressing dendritic cells maturation, while inducing anti-inflammatory cytokines. To identify additional immuno-therapeutic targets that may have activity when used in combination with PS targeting antibodies, we employed bioinformatics analysis on tumors from murine triple negative breast cancer (TNBC) treated with PS and PD-1 targeting antibodies, alone or in combination. Interestingly, expression of the lymphocyte activation gene 3 (LAG3) increased in each treatment arm, suggesting it may be a potential target for combinational therapy in breast cancers. To test this hypothesis, immune competent mice harboring the murine TNBC line E0771 were treated with a PS targeting antibody or a LAG3 targeting antibody as single agent or in combination. Our data demonstrate that while PS-blocking and anti-LAG3 therapies each have efficacy in E0771 as single agents, combinational treatment significantly improved growth inhibition and was capable of increasing TILs, including CD8 + and CD3 + T-cells, while reducing the population of MDSCs. Overall, our data suggest that LAG3 targeting may also represent a viable option for the treatment of breast cancer and that the addition of PS targeting antibodies to LAG3 therapy can effectively increase the anti-tumor and immune-activating effects mediated by additional T-cell checkpoint therapies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-13

Title: Difference of immune microenvironment between primary and recurrent tumours in breast cancer patients

Ogiya R, Niikura N, Kumaki N, Bianchini G, Kitano S, Iwamoto T, Hayashi N, Yokoyama K, Oshitanai R, Terao M, Morioka T, Tsuda B, Okamura T, Saito Y, Suzuki Y and Tokuda Y. Tokai University, School of Medicine, Isehara, Kanagawa, Japan; Ospedale San Raffaele, Milan, Italy; National Cancer Center Hospital, Chuo-ku, Tokyo, Japan; Okayama University Hospital, Okayama, Japan and St. Luke's International Hospital, Chuo-ku, Tokyo, Japan.

Body: Background

Immune checkpoint therapy only benefits a fraction of patients, thus huge efforts have been made to develop predictive biomarkers to identify those patients. Immune biomarkers like PD-L1 expression are extremely dynamic and the timing of evaluation, on primary or metastatic disease, may be critical. We have already shown that tumour-infiltrating lymphocytes (TILs) decrease during metastatic progression in triple-negative (TN) and human epidermal growth factor-2 positive (HER2+) breast cancers (Ogiya R, ASCO 2015), suggesting that mechanisms of immune escape contribute and favour the metastatic progression. In this work we aimed to characterize the modulation and changes of specific immune markers during the metastatic spread comparing paired samples from primary and recurrent breast cancers.

Methods

We retrospectively identified 25 patients with HER2+ (n = 14) and TN (n = 11) early breast cancer diagnosed between 1990 and 2009 at Tokai University Hospital, and who subsequently experienced a first regional or distant recurrence confirmed by tumour biopsy/resection. Haematoxylin and eosin-stained slides of these paired samples were evaluated for stromal TILs. Immunohistochemical staining was performed using primary antibodies against CD4, CD8, Foxp3, PD-L1, PD-L2, and HLA-class I.

Results

The sites of first recurrence was the skin (n = 7), brain (n = 6), lymph node (n = 4), lung (n = 3), bone (n = 2), and one of each of bone marrow, liver and muscle. Immunohistochemical evaluations could not be performed in 5 primary tumours and 2 recurrent tumours because of the small quantity of the specimens. The percentage of CD8⁺ T cells staining in the primary tumours was significantly higher (median 16%) than that in recurrent tumours (median 10%) (paired t-test, p = 0.008) Similarly, the percentage of CD4⁺ T cells staining in the primary tumours was significantly higher (median 40%) than that in recurrent tumours (median 25%) (p = 0.026). The percentage of Foxp3⁺ T cells was low (<10%) and similar in both primary and recurrent tumours (p = 0.16). PD-L1, PD-L2, and HLA class I antibody expression was not statistically different between primary and recurrent tumours, but conversions from positive to negative and vice versa were observed. PD-L1⁺ staining (≥1%) was 90% and 85% in primary and metastatic tumours, respectively.

Comparison of positivity rate between primary and recurrent tumours for each antibody

	Primary tumour	Recurrent tumour	P
Total breast tumours (N)	20	23	
TILs positivity rate, median (%)			
CD4	40%	25%	.03
CD8	16%	10%	.01
Foxp3	<10%	<10%	.16
Expression in tumour cells (N)			
PD-L1			
Strong	8	5	.46
Weak	10	15	
Negative	2	3	
PD-L2			

Strong	6	9	.78
Weak	10	11	
Negative	4	3	
HLA			
Strong	4	6	.89
Weak	14	15	
Negative	2	2	

Conclusions

Tumours at first metastatic recurrence in HER2+ and TN breast cancers have a lower percentage of both CD8+ and CD4+ T cells compared to primary tumours, confirming a potential role of immune escape in tumour progression. Other immune markers, including PD-L1, were not found to change significantly, but negative/positive conversions were observed. This suggests that an evaluation of disease at the time of immunotherapy administration might be more informative. These findings warrant larger confirmation studies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-14

Title: T cell profile associated with regional lymph node progression in breast cancer

Adhikary S, Guo Y, Sieling PA A and Lee DJ J. John Wayne Cancer Institute, Santa Monica, CA.

Body: In 2015, the FDA has approved use of seven immune therapies for cancer, an unprecedented level of approval. Most of these immune therapies involve elicitation of a T cell response against the tumor. In order to advance these therapies into additional tumor types, we must gain a better understanding of the role T cells play in antitumor immunity and immune surveillance. There is still a dearth of information on the role of T cells in human breast and breast cancer to allow immune therapies to have the success in breast cancer as in other tumors. One of the limiting factors in studying breast T cells is the availability of sufficient biopsy tissue from the excision of primary tumors. In this study using a 3-D tissue explant approach, previously described for skin T cells, we expanded tumor infiltrating T cells from fresh breast cancer tissue and examined phenotypic and functional qualities of breast T cells using multiparameter flow-cytometry and multiplex Luminex assay. Examination of the expanded breast tumor infiltrating T cells showed presence of both CD4 and CD8 T cells with CD4 T cells at slightly higher frequency (means CD4 54% vs. CD8 35%, $p=0.07$). Analysis of memory populations utilizing expression of the memory markers CD45RO and CD62L revealed the dominant phenotype to be the effector memory (EM) subtype (CD45RO+, CD62L-) in both CD4 and CD8 compartments (means CD4 69% and CD8 65%). When samples were grouped by clinical information, we found that the T cell profile from breast tissue of breast cancer patients showed a distinct T cell profile associated with regional lymph node progression (LN+) vs. disease localized to the primary site (LN-). We found that the frequency of effector type CD8 T cells (EFF) (CD45RO-CD62L- subtype) was higher in breast tumor tissue of patients without lymph node metastasis (LN-) than those with metastasis (LN+) (means 32% vs 12%, $p= 0.01$). Similar pattern was observed in CD4 compartment, although the overall frequency of CD4 EFF cells was much lower than CD8 (means 5% vs 1%, $p= 0.04$). Analyses of culture supernatant of the expanded T cells via multiplex Luminex assay showed robust production of cytolytic granule proteins at basal state. Levels of granzymes A and B were significantly higher in culture supernatants of breast tumor infiltrating lymphocytes of LN- patients compared to LN+ patients (3815 vs 1645 pg/mL, $p=0.005$, 500 vs 268 pg/mL, $p=0.04$, respectively) while there was no difference in the levels of perforin. More importantly the frequencies of total CD4 or CD8 T cells were not significantly different in breast tumor tissue of LN- vs LN+ patients (means CD4 57% vs. 51%, $p=0.6$, CD8 30% vs. 40%, $p=0.3$). Lymph node status is the single most important indicator of disease-free survival and overall survival in breast cancer. Our observation of a distinct T cell profile associated with lymph node metastasis strongly suggests that our 3-D explant method of T cell expansion could serve as an important profiling / prognostic tool. Furthermore, our finding of higher levels of granzymes associated with LN- disease status begs consideration of including these molecules during selection of T cells for adoptive T cell therapy in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-15

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-16

Title: CD103+/PD-1+ T-cells identify a subset of triple-negative breast cancer eligible for targeted checkpoint inhibition

Bottai G, Loi S, Sotiriou C, Roncalli M, Pusztai L, Reis-Filho JS S and Santarpia L. Humanitas Clinical and Research Center, Milan, Italy; Peter MacCallum Cancer Centre, Melbourne, Australia; Institut Jules Bordet, Brussels, Belgium; Humanitas University, Milan, Italy; Yale Cancer Center, New Haven and Memorial Sloan-Kettering Cancer Center, New York.

Body: Background: The presence of tumor-infiltrating lymphocytes (TILs) is a prognostic factor in triple-negative breast cancer (TNBC). In particular, cytotoxic CD8+ cells are associated with favorable outcome in TNBC. Recently, the integrin CD103 have been suggested as a potential prognostic marker in a small cohort of triple-negative/basal-like breast cancer (Wang et al, *Clin Cancer Res* 2016). In this study, we aimed to evaluate the functional status and clinical relevance of CD8+ and CD103+ TILs in TNBC.

Methods: A large collection of formalin-fixed, paraffin-embedded tissues was retrospectively collected from 210 patients with primary invasive ductal TNBC. The presence of CD8, CD103 and the checkpoint receptor PD-1 was assessed by immunohistochemistry. Whole tumor slides were independently assessed by two pathologists blinded for patient characteristics and outcome. Cases where $\geq 5\%$ of TILs expressed CD103 or PD-1 were considered positive. Statistical analyses were performed using Spearman's correlation, Kaplan-Meier and Cox regression analyses.

Results: We found that CD103+ cells mostly co-expressed the CD8 marker, and were preferentially distributed within tumor epithelium. No consistent correlation was found between the presence of CD103+ lymphocytes and the expression of its ligand E-cadherin by TNBC cells ($rs=0.365$). CD8+ lymphocytes were consistently associated with better relapse-free survival (RFS) and overall survival (OS) in both univariate (HR=0.61; $P=1.06E-02$ for RFS; HR=0.59; $P=1.93E-02$ for OS) and multivariate analysis (HR=0.65; $P=2.45E-02$ for RFS; HR=0.63; $P=3.96E-02$ for OS). The presence of CD103+ lymphocytes significantly correlated with prolonged RFS and OS in univariate analysis only (HR=0.82; $P=4.12E-02$ for RFS; HR=0.85; $P=4.93E-02$ for OS), and a trend for longer RFS was also observed in univariate analysis for PD-1 (HR=0.71; $P=5.22E-02$). Interestingly, a subset of TNBC showed co-expression of CD103+/PD-1+ in lymphocytes localized to the intraepithelial areas of the tumor.

Conclusions: The expression of CD103 on CD8+ T cells in direct contact with cancer cells may identify a subpopulation of cells with potent cytolytic activity. The interaction between CD103+ cytotoxic lymphocytes and cancer cells is mediated by mechanisms other than the binding to E-cadherin. Even though CD103 has a role in mediating an effective anti-tumor immune response, its presence alone may not be sufficient to impact the outcome of TNBC patients. Immunomodulatory therapies may be useful to boost the anti-tumor activity of potentially quiescent CD103+/PD-1+ cells in a subgroup of TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-17

Title: Directing NK cells to Trop-2-expressing breast and other cancers, with chimeric antigen receptors

Chang C-H, Liu D and Goldenberg DM M. Immunomedics, Inc., Morris Plains, NJ.

Body: Adoptive immunotherapy with chimeric antigen receptor (CAR)-engineered T or NK cells is in active clinical and preclinical development, with recent interest increasingly focused on advancing the availability of off-the-shelf allogeneic clonal NK cell lines capable of targeting tumors with appropriately designed CARs. Among the human NK cell lines established, the IL-2-dependent NK-92 is the most studied and has been transduced with human IL-2 to generate NK-92MI, which exhibits cytotoxicity similar to NK-92, and is independent of IL-2 for its cytotoxicity, viability and proliferation. We report the assembly of E1.BB.3z-92MI, a novel CAR-NK for potential therapy of diverse Trop-2-expressing breast, lung, bladder, ovarian, and other cancers. The Trop-2-specific CAR, referred to as hRS7-CAR, consists of the CD8 α signal peptide, the V_K and V_H of hRS7 (a humanized anti-human Trop-2 mAb used in the antibody-drug conjugate, IMMU-132), the hinge region and transmembrane domain of CD8 α , the intracellular domains (ICD) of 4-1BB, and the ICD of CD3 ξ . The construct of hRS7-CAR is introduced into NK-92MI either by electroporation of the mRNA synthesized in vitro, or by inoculation with the lentiviral particles harvested from the 48-h supernatants of Lenti-X 293T cells transduced with pLVX-puro-hRS7-CAR.

The presence of hRS7-CAR in E1.BB.3z-92MI was probed by Western blot using an HRP conjugate of a rat anti-id antibody against hRS7, which detected a distinct band of about 50 kDa from the cell lysates of NK-92MI transfected with hRS7-CAR mRNA, but not from the mock-transfected NK-92MI. As the calculated molecular weight of hRS7-CAR is about 51 kDa, these results support that hRS7-CAR is produced in NK-92MI cells transfected with hRS7-CAR mRNA. Additional evidence by flow cytometry shows about 41% of NK-92MI cells transfected with hRS7-CAR mRNA are alive at the time of analysis and 25% of this subpopulation expresses hRS7. The cytotoxicity of E1.BB.3z-92MI was evaluated against the Trop-2-expressing, human breast cancer cell line, HCC1806, in 96-well plates (4,500 cells/well) at 3 different effector-to-target (E/T) ratios (1:1, 2:1, or 4:1). As shown in the Table, the results of the MTS assay indicate that NK-92MI cells transfected with hRS7-CAR mRNA significantly killed more HCC1806 cells at the E/T ratio of 2:1 or 4:1, in comparison to mock-transfected NK-92MI. Another study performed by flow cytometry using dye-labeled HCC1806 at the E/T ratio of 3:1 indicates that the specific lysis of HCC1806 cells by NK-92MI cells transfected with hRS7-CAR mRNA was about 2-fold higher than that observed for mock-transfected NK-92MI. Finally, the initial results obtained for NK-92MI cells transduced with the lentiviral pLVX-puro-hRS7-CAR show about 70% viability and greater than 30-fold higher MFI in the live population than the control transduced with pLVX-puro or not transduced. Together, these results demonstrate the feasibility of developing E1.BB.3z-92MI as a novel CAR-NK for targeting Trop-2-expressing cancers, including breast cancer.

Table. Cytotoxicity of Trop-2-targeting NK cells

E/T	Viability (mean \pm SD)		Mock vs. hRS7-CAR	
	Mock	hRS7-CAR	Δ	P-value
1:1	88.5 \pm 9.9	80.3 \pm 11.1	8.2	0.39
2:1	89.9 \pm 10.6	67.4 \pm 4.4	22.5	0.027
4:1	84.8 \pm 4.0	72.4 \pm 2.5	12.4	0.010

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-18

Title: JAK2 and PD-L1 amplification enhance the dynamic expression of PD-L1 in triple negative breast cancer

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Body: Background: Triple negative breast cancer (TNBC) is a heterogeneous disease. Amplification of chromosome 9p24.1 encoding *JAK2* and *PD-L1* has been reported in up to 25% of TNBC and is associated with poor clinical outcome. In lymphoma, *JAK2* is a transcriptional activator of both PD-1 ligands, and chromosome 9p copy number gain has been associated with therapeutic activity of nivolumab. We evaluated the interaction of *JAK2* and *PD-L1* expression in TNBC.

Methods: 9p24.1 amplification in 4 TNBC cell lines (MDA-MB-231, MDA-MB-436, HCC1937, and HCC70) was measured using array comparative genomic hybridization (aCGH). Amplification was defined as aCGH \log_2 ratio > 2.0. Cell surface expression of *PD-L1* was detected by flow cytometry and compared with the median fluorescence intensity (MFI) of isotype control Ig. To selectively inhibit *JAK2*, lentiviral vectors encoding two different shRNA were generated. *JAK2*, pSTAT1 and pSTAT3 expression were measured by immunoblot. The effects of the anti-*JAK1/2* inhibitor ruxolitinib and interferon-gamma (IFN- γ) induction of *PD-L1* was measured.

Results: 9p24.1 copy number loss was measured in MDA-MB-231 (\log_2 ratio = -1), neutral in HCC1937 (\log_2 ratio = 0), gained in MDA-MB-436 (\log_2 ratio = +1) and amplified in HCC70 (\log_2 ratio = +2). No correlation was observed between *PD-L1* expression and the 9p24.1 amplification, with the MFI ratio range from 5 to 16.5, mean 8.3 by flow cytometry. TNBC cell lines had higher baseline expression of *PD-L1* compared to the ER+ cell lines T47D and MCF-7 (ratio, 0 p=0.1). Low dose IFN- γ (1.0-10.0 ng/ml) rapidly induced expression of *PD-L1* in MDA-MB-231 (1.5 fold increase) and HCC70 (3.3 fold increase) with significant activation of the *JAK2/STAT1* pathway. The induction of pSTAT1 and *PD-L1* expression by IFN- γ was blocked with low dose (1mM) *JAK1/2* inhibitor ruxolitinib. Knockdown of *JAK2* with shRNA (>80%) did not impact *PD-L1* baseline expression in MDA-MB-231 and HCC70 but abrogated IFN- γ -mediated induction of *PD-L1* and the phosphorylation of STAT1.

Conclusion: These data suggest that TNBC cell lines have baseline *PD-L1* expression, but the cells with 9p24.1 amplification are highly sensitive to *PD-L1* induction with IFN- γ which can be abrogated with inhibition with a *JAK1/2* inhibitor or shRNA. Synergistic inhibition of *PD1/PD-L1* and *JAK2* may have therapeutic efficacy in the subset of TNBC with 9p24.1 amplification, and the dynamic effects of tumor *PD-L1* expression in response to local inflammation should be considered in the evaluation of *PD-1/PD-L1* checkpoint blockade.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-19

Title: Elucidating the tumor immune microenvironment phenotype in early stage untreated BRCA mutated breast cancer patients

Force J, Abbott S, Broadwater G, Kimmick G, Westbrook K, Hwang S, Kauff N, Stashko I, Weinhold K, Nair S, Hyslop T, Blackwell K, Castellar E and Marcom PK. Duke University, Durham, NC; Duke University, Durham, NC; Duke University, Durham, NC and Duke University, Durham, NC.

Body: Background: Increased stromal tumor infiltrating lymphocytes (TILs) are predictive and prognostic for improved outcomes from neoadjuvant or adjuvant chemotherapy in triple negative breast cancer. Increased tumor mutational burden may promote neoantigens causing immune system upregulation. Microsatellite instability in gastrointestinal cancer predicts for response to checkpoint inhibition and is associated with inherited cancer predisposition. The immune system response in BRCA mutated breast cancer has not been described. The purpose of this study is to assess tumor infiltrating immune cells in early stage breast cancer patients with and without BRCA gene mutations.

Methods: We retrospectively investigated 124 early stage breast cancer patients with BRCA mutations (n=62, BRCA+) and without BRCA mutations (n=62, BRCA WT). The %TILs was measured manually by H&E. Our control group consisted of age, stage, and receptor status matched early stage untreated breast cancer patients who were deemed BRCA WT by extended gene panel testing or were negative for BRCA 1/2 and had a posttest probability of harboring an autosomal dominant mutated gene of $\leq 1\%$ using the Bayes-Mendel algorithm. We used a two-sample binomial arcsin approximation to detect a 20% difference in TILs between cohorts to attain 80% power with a one-side alpha of 0.05. Wilcoxon Rank-Sums test was used to compare differences in the central tendencies for continuous variables. We used the Nanostring PanCancer immune profiling panel to immunophenotype a portion of the BRCA+ and BRCA WT cohorts and used nSolver for quality control, normalization, and bioinformatics analyses.

Results: Here we report TILs from the first 21 patients of our study. Thirteen patients harbored BRCA mutations and eight patients did not. All patients were HER2 negative. Eight (61%) and four (50%) patients were hormone receptor positive (HR+) in the BRCA+ and BRCA WT cohorts, respectively. Median %TILs were not significantly different between the BRCA+ (15, range 0-70) and BRCA WT (17.5, range 5-60; $p=0.7$) groups. Median %TILs in the HR+/BRCA+ (12.5, range 0-50) and HR-/BRCA+ (15, range 5-70) cohorts were not statistically different when compared to HR+/BRCA WT (10, range 5-15; $p=0.4$) and HR-/BRCA WT (30, range 20-60; $p=0.2$) cohorts, respectively. There were 2 patients with lymphocyte predominant breast cancer (n=1, HR-/BRCA+; n=1, HR-/BRCA WT).

Conclusions: This is the first study to characterize TILs and a tumor immune microenvironment phenotype in early stage breast cancer patients with BRCA mutations. These results suggest harboring a BRCA mutation is not associated with increased TILs in early stage untreated breast cancer patients. This conclusion stayed true regardless of hormone receptor status. However, a trend of decreased TILs was seen in HR-/BRCA+ patients when compared to those with HR-/BRCA WT disease. Moreover, the median and range of TILs were higher in the HR+/BRCA+ group compared to the HR+/BRCA WT group. This suggests increased TILs may exist in some HR+ patients with a BRCA mutation. Further investigation of TILs and immune profiling of early stage untreated breast cancer patients with and without BRCA mutations is warranted.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-20

Title: PD-L1 expression in triple negative breast cancer (TNBC) is associated with improved outcomes

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Body: Background: Breast cancer (BC) evolution is influenced by tumor microenvironment. Presence of CD8+ cytotoxic T lymphocytes (TILs) has been proposed as surrogate marker of adaptive immune response, and programmed death ligand-1 (PD-L1) is a negative regulator of the tumor immune microenvironment. However, whether PD-L1 expression adversely affects breast cancer outcome is unknown (*Oncotarget* 2014; 6:5449). Tumor-associated macrophages (TAMs) in the tumor microenvironment may contribute to BC progression and metastagenicity. We assessed the potential correlations between PD-L1 expression, the presence of TAMs and TILs, and BC outcomes.

Methods: 59 primary BCs (16 HR+, 16 HER2+, and 27 TNBC) with known clinical and pathological features and patient (pt) follow-up for a median of 3.9 years were evaluated by immunohistochemistry for expression of CD8, CD68, and PD-L1 within tumor and stroma. The average number of CD8+ cells within 10 high power fields was determined separately for invasive tumor cell nests and for stroma within each sample, and the median number of CD8+ cells within tumor vs stroma was calculated (*Breast Cancer Res Treat* 2011 128:703–711). Non-lymphocyte mononuclear cells in tumor and stroma were used in counting CD68+ TAMs. BCs were positive for CD8+ TILs (Cell Marque clone #C8144B) or CD68+ TAMs (Cell Marque clone #KP1) if the number of cells positive in the sample was greater than the median. PD-L1 (Dako 28-8 pharmDx) was positive if at least 1% of tumor cells expressed PD-L1. The log-rank was used to compare the survival and progression free survival between groups and Spearman's rank-order correlation tests were conducted to determine associations between CD8, CD68, and PD-L1.

Results: 57% of TN, 26% of HER2+ and 13% of HR+ BCs expressed PD-L1 in tumor. TNBC pts received anthracycline/ taxane chemotherapy (62%) or taxane therapy alone (22%). HR+ and HER2+ pts received standard endocrine therapy and trastuzumab-based therapy. Stromal CD8+ TILs were associated with improved OS in the overall population (log rank $p=0.026$). In TNBC, PD-L1 expression was positively correlated with the presence of TILs ($p=0.0002$) and TAMs ($p=0.0005$) as well as with improved 3 year PFS and OS (log rank $p=0.04$ and $p=0.03$, respectively). Furthermore, stromal CD8+ TILs and stromal CD68+ TAMs correlated positively with each other in the TNBC group ($p=0.0094$).

Conclusions: PD-L1 expression correlated with TILs and TAMs in TNBC and was associated with a favorable outcome. PD-L1+ TNBCs with high levels of TILs and TAMs may be primed for exceptional response to immunogenic chemotherapy alone. Whether some pts with PD-L1+ TNBCs with high TILs/TAMs will benefit additionally from an anti-PD-L1/PD-1 agent is being investigated currently.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-21

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-22

Title: Porous silicon microparticle potentiates anti-tumor immunity

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Body: Cancer genome sequencing and new technologies to identify immunogenic tumor mutations have offered an unprecedented opportunity to target tumor-specific neoantigens for effective cancer treatment with the vaccination strategy. However, lack of potent adjuvants to effectively stimulate antigen-presenting cells and promote sustained antigen processing and presentation in the immunosuppressive microenvironment has severely limited therapeutic cancer vaccine development. Our recent study has shown that porous silicon microparticle (PSM) is an ideal adjuvant for therapeutic cancer vaccines (*Xia et al: Cell Reports 2015, 11:957-966*). PSM can 1) stimulate expression of type I interferons in dendritic cells through TRIF/MAVS-dependent signal transduction pathways leading to dendritic cell maturation, 2) synergize with soluble adjuvants to promote dendritic cell maturation, 3) facilitate intracellular antigen transport and mediate antigen processing in lysosomes and proteosomes, 4) enhance dendritic cell accumulation in secondary lymphoid tissues of tumor-bearing mice, and 5) serve as a reservoir for sustained release of tumor antigen and soluble adjuvants. Treatment of murine models of HER2 positive breast cancer with a PSM-based therapeutic cancer vaccine activates and expands antigen-specific CD8+ T cells and promotes a Th2-to-Th1 transition in the tumor microenvironment, leading to potent inhibition of tumor growth and metastasis. In summary, PSM-based cancer vaccine is a promising immunotherapeutic agent for effective treatment of human cancers including cancer types that are traditionally considered as weakly immunogenic.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-23

Title: A monoclonal antibody against hypo-glycosylated bone sialoprotein II has application for diagnostic purposes in samples of breast cancer patients and for treatment of skeletal metastasis caused by MDA-MB-231 breast cancer cells in rats

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Body: The SIBLING protein bone sialoprotein II (BSP) has been implicated in lytic skeletal metastasis as it is expressed in a subset of primary breast cancers and can be detected at elevated levels in the serum of patients with increased risk to develop skeletal metastasis. The aim of this study was to investigate the potential application of a rat monoclonal antibody against hypo-glycosylated BSP (IDK1) for diagnostic and therapeutic purposes.

The diagnostic part of this study was based on breast cancer specimens from the biobank / repository of the Institute of Pathology of the Municipal Hospital Kassel, Germany. Immune-histochemical analyses were performed with IDK1 for comparing BSP expression between ten human primary breast tumor sections and their corresponding bone metastatic tissue samples. The therapeutic part of this study was based on a model in nude rats, in which the rats were implanted with human MDA-MB-231 breast cancer cells for selective and orthotopic appearance of osteolytic skeletal lesions. Tumor bearing rats were treated with IDK1 starting at two or four weeks after tumor cell inoculation into the femoral artery of one hind leg. Tumor growth was monitored by light emission, caused by luciferase mediated metabolism of luciferin. Photon emission was recorded at regular intervals by a Xenogen IVIS 100 imaging system. After sacrifice, samples of lesions and apparently healthy tissues were investigated by H&E staining as well as by immune-histological staining for BSP.

BSP staining was found within the cytoplasm of tumor cells. Increased expression of BSP was also detected in healthy bone cells, e.g. osteoblasts, as soon as breast tumor cells invaded bone tissue. An elevation of BSP expression near necrotic centers was also found. Expression of BSP in primary breast tumors was positively correlated with BSP expression in bone metastases. Furthermore, bone metastases showed higher and more intensive expression of BSP than their respective primary breast tumors ($p < 0.0039$).

In the experimental treatment part, all but one untreated tumor bearing rats showed rapid tumor growth accompanied with lytic destruction of femur and tibia of the respective hind leg (18/19; tumor take rate 95%). In contrast, rats treated with the anti-BSP antibody did not show a significant increase in light emission nor a clinical deterioration. In fact, 8 of 10 rats receiving the antibody at a dose of 10 mg/kg/week starting at two weeks after tumor implantation did not show any light emission after 4 to 6 weeks ($p = 0.01$ versus control) as well as 6 of 10 rats receiving the antibody at the same dose starting at four weeks after tumor implantation ($p < 0.05$). Radiological and histological examination confirmed that animals without light emission were free of tumor growth, corresponding to a complete remission.

In conclusion, the rat monoclonal antibody directed against BSP is a powerful tool with potential for diagnostic and therapeutic applications in breast cancer skeletal metastasis and warrants further development.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-24

Title: Changes of tumor-infiltrating lymphocytes and programmed death-ligand1 positivity after neoadjuvant chemotherapy in patients with locally advanced breast cancer

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Body: Background

Recently, the balance in immune system between immune surveillance and tolerance is known to be associated with the prognosis of breast cancer patients. The aim of this study was to investigate changes of tumor-infiltrating lymphocytes (TILs) and programmed death-ligand1 (PD-L1) status after neoadjuvant chemotherapy (NAC) and their impact on recurrence in patients with locally advanced breast cancer.

Methods

Paired samples (before and after NAC) of triple negative or HER2+ breast cancer tissue were obtained from clinical stage II or III patients (n=39) undergoing NAC and subsequent breast resection. The assessment of immunohistochemical (IHC) staining for PD-L1 were performed. Immunostaining of forkhead box P3 (Foxp3) and CD4/CD8 were performed for subtyping of TILs in triple negative breast cancer (TNBC) only. Clinicopathologic data including baseline characteristics, tumor response and recurrence were reviewed.

Results

Proportion of PD-L1 (+) tumor cells in pre-chemotherapy tissue was 20% (5/25) in HER2+ and 28.6% (4/14) in TNBC. It could not predict pathologic complete response. Most post-chemotherapy tissue (21/24) showed same PD-L1 positivity with pre-chemotherapy tissue. The rest showed the decreased PD-L1 positivity after NAC.

In cases of TNBC, the increased number of CD8+ T cells was significantly associated with PD-L1 positivity in pre-chemotherapy tissue. ($p=0.001$) Foxp3+ T cell proportions decreased significantly ($p=0.046$) and CD8+/Foxp3+ T-cell ratio increased significantly ($p=0.023$) after NAC. The patients with increased number of CD8+ T cells after NAC had a tendency to live longer without recurrence compared to patients with decreased CD8+ T cells (62.3 vs 38.1 months, $p=0.158$).

Conclusion

Our data provides the clinical evidence that PD-L1 positivity are associated with CD8+ T cell proportion and increased CD8+ T cells after NAC might be good prognostic marker. The role of immunologic balance as a prognostic marker for recurrence must be evaluated in future study.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-25

Title: Breast cancer patients after kidney and liver transplantation: A Report from Asan medical center

Jung I and Kim HJ. Asan Medical Center, College of Medicine, University of Ulsan, Seoul, Song Pa, Republic of Korea.

Body: Background: Improvements in immunosuppressant and operation have resulted in long life expectancy of transplantation. Immune mechanism becomes important issue while cancer development and progression. This study was to evaluate the nature of post transplant breast cancer (PTBC) compared with breast cancer in general population, and to evaluate the relationship between immunosuppression and breast cancer development

Methods: Using information from three asan medical center databases between 1989 and 2014, including asan medical center breast cancer (AMCBC) database, Kidney transplantation database and liver transplantation database, two cohort composed of PTBC cohort and normal breast cancer control cohort. Survival were compared the patients who received breast cancer operation before 2008.

Results:

After median 58.3 month after liver transplantation, 10 patients were diagnosed as breast cancer and 23 patients after median 108.3 month kidney transplantation ($p < 0.001$) Mean age of breast cancer were $53.2 (\pm 8.5)$ years old in liver transplantation patients and $44.2 (\pm 4.8)$ years old in kidney transplantation patients. The age in kidney transplantation patients was significantly younger than general breast cancer population (44.2 ± 4.8 years old, 47.52 ± 10.0 years old respectively, $p = 0.006$). Asymptomatic screening were the detection method of 33.4% of PTBC cohort but 21.0% of control cohort ($p < 0.001$) On PTBC cohort, 21.3% (10 patients) were stage 0 breast cancer compared with 9.4% (1002 patients) in control cohort ($p < 0.001$), 90% (28) patients were lymph node negative tumor compared with 62.9% (6669 patients) in control group ($p < 0.001$). Estrogen receptor, Progesteron receptor, Her2 positivity were not difference between cohorts. On multivariate analysis, immunosuppressant is not a poor prognostic factor for breast cancer patients (HR 1.319 95%CI 0.329-5.287).

Conclusion

The age of breast cancer diagnosis was younger in the patients who received kidney transplantation. PTBC patients have early breast cancer due to early detection and prognosis was comparable compared with general breast cancer patients. Immunosuppressants does not adversely affect breast cancer prognosis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-26

Title: Identifying patient-specific neoepitopes for cell-based and vaccine immunotherapy across breast cancer classifications reveals rarely shared recurrent neoepitopes

Nguyen A, Sanborn JZ, Vaske CJ J, Rabizadeh S, Niazi K, Soon-Shiong P and Benz SC C. NantOmics LLC, Santa Cruz, CA; NantOmics LLC, Culver City, CA and Chan Soon-Shiong Institute of Molecular Medicine, Culver City, CA.

Body: Introduction: Targeted therapies for breast cancers such as trastuzumab and everolimus have durable clinical benefits for patients that express the relevant biomarkers (HER2 and mTOR respectively). Triple negative breast cancer patients lack these biomarkers and are left with few options. Recent advances in immunotherapy agents against PD-1/CTLA4 for patients with melanoma have yielded amazing clinical benefits for a subset of patients and may have similar results in breast cancer patients, but again the vast majority of patients still undergo disease progression. We analyzed whole genome sequencing (WGS) and RNA sequencing data from The Cancer Genome Atlas (TCGA) to identify neoepitopes among breast cancer patients that could be used to develop next-generation, patient-specific cancer immunotherapies. Neoepitopes are tumor specific markers that arise from mutations acquired from cancer and may represent a path to targeted therapies even in triple negative breast cancers.

Results: We analyzed 99 breast cancer patients from TCGA, containing a mixture of PR+/HER2+/ER+ and TNBC classifications. These breast cancer patient samples were selected by the availability of whole genome sequencing (WGS) data, RNA-sequencing data as well as clinical outcome data. We identified an average of 680 potential neoepitopes per patient based solely on WGS data. To further refine and select high quality neoepitopes we restricted these neoepitopes based on gene expression yielding an average of 304 expressed neoepitopes per patient. We predicted each patient's HLA typing using only omics data, which we then used to predict HLA-expressed neoepitope binding analysis resulting in an average of 11 high-quality tumor specific neoepitopes per patient. We identified few recurrent neoepitopes that were bound and expressed, indicating the need for a personalized medicine approach.

Conclusions: Within the TCGA dataset, the majority of neoepitopes among patients with breast cancer were unique to each patient. Rarely within subsets of breast cancers such as HER2+, we identify neoepitopes that are shared between patients. For breast cancer patients who do not respond to targeted therapies, high-throughput identification of neoepitopes could serve as the basis for the development of next-generation, patient-specific immunotherapies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-04-27

Title: CTLA-4 and PD-1 checkpoint inhibitors enhance individually tailored adaptive anti-tumor immune responses to overcome tumor immunosuppression and effectively treat triple-negative breast cancer

Hartman ZC C, Crosby EJ J, Wei J-P, Yang X-Y, Lei G-J, Wang T, Liu C-X, Agarwal P, Morse MS S and Lyerly HK K. Duke University, Durham, NC.

Body: Despite a lack of unifying drivers in Triple-Negative Breast Cancer (TNBC), our lab and others have uncovered that these cancers have elevated expression of inflammatory genes and immunosuppressive molecules (i.e. PD-L1), as well as elevated numbers of infiltrating immune cells (including CD8+ T-cells and Foxp3+ T-regulatory cells) which suggests the therapeutic potential for single and combinations of checkpoint blockade antibodies. While early trials with PD-1 inhibitors have been encouraging for TNBC, only a fraction of treated patients respond to this therapy. To test and define the mechanisms that govern responses, we explored the utility and mechanistic basis of both PD-1 and CTLA-4 inhibition in generating tumor-specific immunity in an established murine model of TNBC.

Consistent with patient samples, we found TNBC tumors from our model exhibited elevated PD-1+ expressing CD8+ T-cell infiltrates, Foxp3+ T-regulatory cell infiltrates (~66% of CD4+ TILs), as well as highly elevated tumor cell expression of PD-L1. We also found that while TNBC cells were easily killed by T-cell in vitro, TNBC tumors were highly immuno-suppressive and resistant to antigen-specific T-cell attack in vivo, even after adoptive transfer of up to 5x10E6 tumor-specific T-cells.

However, we found that both CTLA-4 and PD-1 antibodies could curtail this immunosuppression to different degrees and through alternate mechanisms. Specifically, we found that CTLA-4 antibody mediated anti-tumor immunity through the elimination and blockade of Foxp3+ T-regulatory cells in the tumor microenvironment, which allow for potent T-cell expansion. Conversely, PD-1 antibodies elicited anti-tumor immunity through blockade of PDL1/PD1 signaling between tumor cells and T-cells in the TNBC tumor microenvironment that allowed for a more modest expansion of individually tailored T-cell specific clones in vivo.

Strikingly, the combination of these antibodies and their alternate mechanisms of action resulted in greatly enhanced anti-tumor responses and led to regression of ~80% of tumors. This was accompanied by an augmented infiltration of T-cells into the tumor microenvironment and significantly enhanced systemic tumor-specific T-cell responses, which appear to be emergent properties of dual CTLA-4/PD-1 antibody treatment.

However, we found that these treatments did not expand a common tumor-specific T-cell clone, despite adoptive transfer of identical tumor-specific immunodominant T-cells into mice after tumor implantation. Thus, despite our use of a highly homogeneous model utilizing genetically identical mice implanted with an identical tumor line bearing a unique tumor antigen under identical conditions, the tumor-specific T-cell responses were highly unique for each individual tumor. Collectively, our study suggest that dual blockade could be an effective therapeutic clinical strategy against TNBC and further suggest the utility of monitoring systemic immune response and TCR expansion of TILs as the most useful correlates in clinical studies utilizing CTLA-4 and PD-1 antibodies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-01

Title: Clinico-pathological relationships with Ki67 in POETIC (CRUK/07/015) – Critical lessons for assessing Ki67 for prognosis and as a pharmacodynamic marker

Bliss JM M, Morden J, Evans A, Holcombe C, Horgan K, Mallon E, Raghavan V, Skene A, Dodson A, Hills M, Detre S, Zabaglo L, Graf M, Banerji J, Gillman A, Robertson J, Dowsett M, Smith I and On Behalf of the POETIC Trialists. Institute of Cancer Research, London, United Kingdom; Poole Hospital, Poole, United Kingdom; Royal Liverpool University Hospital, Liverpool, United Kingdom; Leeds General Infirmary, Leeds, United Kingdom; Western Infirmary, Glasgow, United Kingdom; Royal Wolverhampton Hospitals, Wolverhampton, United Kingdom; Royal Bournemouth Hospital, Bournemouth, United Kingdom; Royal Marsden Hospital, London, United Kingdom; Nottingham University Hospitals, Nottingham, United Kingdom and POETIC Trialists, United Kingdom.

Body: Background Higher levels of the proliferation marker Ki67 at breast cancer (BC) diagnosis are increasingly recognised to indicate poorer prognosis. Change in Δ Ki67 in response to endocrine treatment reflects response. The precise relationship between Ki67 and other clinico-path factors and how associations are affected by short exposure to aromatase inhibitor (AI) has been unclear.

Methods POETIC was a UK-wide, phase III, randomised trial which tested perioperative use of AI (anastrozole (A), letrozole (L)) in postmenopausal women with early BC (Dowsett JNCI Monogr 2011). Ki67 was measured in a single central lab at diagnosis (B=baseline) and 2 weeks later at surgery (S) allowing in vivo assessment of AI sensitivity. POETIC recruited 4483 women from 130 UK centres. Paired biopsies were available for 96%. Relationship between Ki67 and clinico-path factors is described by summary statistics (median) and independent associations explored in multivariable linear regression models (MVM). Analyses of Ki67 at S and Δ Ki67 (reduction) were adjusted for B Ki67 and surgical sample type.

Results

Variable	Baseline Ki67 %			Ki67 change (%) with 2 weeks AI		
	N	Median	ln(Ki67) P value (MVM)	N	Median	ln (Ki67(S)/Ki67 (B)) P Value (MVM)
All patients	3913	15.2		2528	77.6	
PgR (local)			0.01			<0.001
Positive	2219	14.6		1411	80.0	
Negative	477	20.4		326	61.1	
Not known	1217	15.1		791	77.0	
HER2 (local)			<0.001			<0.001
Negative	3459	14.3		2240	79.2	
Positive	402	26.8		265	52.9	
Unknown	52	13.9		23	85.5	
Tumour grade (B)			<0.001			<0.001
G1	548	9.1		343	78.6	
G2	2275	14.5		1494	79.6	
G3	703	30.1		447	62.1	
Unknown	387	14.0		244	75.4	
Tumour size (S)			0.002			0.49
<2cm	1503	12.9		983	79.1	
2-5cm	2221	17.5		1425	76.4	
>5cm	174	16.9		114	74.1	

Histological type (B)			<0.001			0.58
Ductal	3138	16.3		2030	77.5	
Lobular	549	11.9		353	79.0	
Other/Not known	226	13.1		145	74.0	
Nodes involved			0.03			0.53
0	2357	14.1		1535	77.6	
1-3	1108	15.8		708	78.2	
4+	433	20.3		278	76.1	
Vascular invasion			<0.001			0.03
Yes	1121	19.9		711	75.9	
No	2603	13.6		1695	78.2	
Unknown	189	14.6		122	75.8	

Absolute Ki67 (B) was associated with each classic prognostic factor. Factors affecting Δ Ki67 in control pts were Ki67 (B), grade (B) and surgical sample type (core cut 4.1%; excision 17.7% $p < 0.001$). After 2 weeks of AI, variation in extent of Δ Ki67 was dominated by PgR, HER2 and grade (B). Centres using L more often supplied excision samples ($p < 0.001$). In spite of this both surgical sample type and choice of AI (both $p < 0.001$) independently influenced Δ Ki67 (core cut: A 65.1% L 73.8% excision A 75.2% L 81.3%). Pattern of change in grade between B and S differed for AI and control.

Summary POETIC provides the largest multi-centre series of women in whom centrally assessed Ki67 has been correlated with classic clinico-path factors and impact of short term AI exposure explored. Choice of AI and surgical sample type are both dictated by participating site and their inter-relationship requires further review. Whether the greater suppression of Ki67 following L, a drug associated with better E2 suppression and aromatisation than A has clinical consequences is beyond the scope of this work. Relationships found (e.g. sample type, grade) are critical for interpretation of studies using Ki67 for prognosis and Δ Ki67 as a pharmacodynamic response marker.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-02

Title: Active surveillance with a combination of tumor marker CA27.29 and detection of circulating tumor cells two year after primary diagnosis strongly predicts subsequent prognosis

Janni W, Rack B, Häberle L, Friedl TWP WP, Tesch H, Lorenz R, Jäger B, Fehm T, Müller V, Schneeweiß A, Lichtenegger W, Blohmer J, Beckmann MW W, Scholz C, Pantel K, Trapp E and Fasching PA A. University of Ulm, Ulm, Germany; University of Munich, Munich, Germany; University of Erlangen, Erlangen, Germany; Onkologische Praxisklinik, Frankfurt, Germany; Onkologische Praxisklinik, Braunschweig, Germany; Heinrich-Heine-University, Duesseldorf, Germany; Universitätsklinikum Eppendorf, Hamburg, Germany; University of Heidelberg, Heidelberg, Germany and Charite University Hospital, Berlin, Germany.

Body: Introduction

The prognosis of patients with early breast cancer is commonly estimated by prognostic factors obtained at the time of the initial diagnosis. However, patients and physicians alike are seeking for factors evaluating the prognosis years thereafter during follow-up. The identification of a patient group with an unfavourable prognosis could lead to secondary treatment intervention, potentially improving outcome. Aim of the study was to assess the added prognostic value of circulating tumor cells (CTCs) and CA27.29 beyond established predictors.

Materials and Methods

Patients of the phase III SUCCESS-A study were included into this analysis (n=1005). SUCCESS-A is a chemotherapy study for high risk patients with a comprehensive translational research program, which included the determination of CTCs and CA27.29 two years after the initial diagnosis. A Cox regression model with disease-free survival (DFS) as outcome and well-established predictors (age, BMI, pT, pN, histology, grading, ER, PR, Her2neu) was compared with an extended Cox model with the well-established predictors and additionally CTC (>0 versus 0) two years after randomization, and CA27.29 (in U/mL) measured after chemotherapy and again two years after randomization using a likelihood ratio test. In case of significance, the extended model was applied to predict for each patient the risk of disease recurrence within the next 12 months (0 to 100%).

Cross-validated AUC, sensitivity and specificity values were determined to assess clinical usefulness of risk prediction.

Results

The markers CA27.29 and CTC were both significantly associated with subsequent prognosis ($p < 0.000001$). The detection of CTCs increased the risk of subsequent DFS events (HR=2.14, 95%CI: 1.31-3.48), while CA27.29 after two years increased the risk for DFS events with a HR of 1.12 per U/mL increase (95%CI: 1.09-1.15). The combination of the two markers significantly empowered the prognostic relevance, with a HR of 6.64 for patients with CTCs and an elevated CA27.29 by 10 U/mL compared to patients without CTCs and without CA27.29 elevation. The mean risk of disease recurrence in the third year after randomization was 2.38%. Discrimination of patients with and without disease recurrence based on risk prediction from the extended Cox model (AUC: 0.80) was better than discrimination based on the clinical model without the markers CTC and CA27.29 (AUC: 0.64). Sensitivity with regard to decision thresholds 1%, 2%, 3%, and 4% predicted risk was 0.89, 0.77, 0.65, and 0.55, respectively. The corresponding specificity was 0.42, 0.69, 0.81, and 0.88.

Discussion

Both CTCs and CA29.27 values determined 2 years after primary diagnosis are clinically relevant predictors of subsequent prognosis for those patients. This study extends evidence for active surveillance of breast cancer survivors. Identifying a group of women with a high recurrence risk after two years could be the basis for the development of secondary adjuvant treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-03

Title: Intra-tumor heterogeneity of the estrogen receptor predicts less benefit from tamoxifen therapy and poor long-term breast cancer patient survival – Retrospective analyses of the STO-3 randomized trial

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Body: Background

We and others have shown that the clinically used breast cancer markers alter their expression throughout tumor progression, influencing patient survival (Lindström et al, JCO 2012). What are the likely explanations to our findings? Here, we aimed to determine whether breast cancer intra-tumor heterogeneity of the estrogen receptor (ER) is a marker of tumor aggressiveness and benefit of tamoxifen therapy in a large randomized trial.

Material and methods

The Stockholm Tamoxifen (STO-3) trial enrolled postmenopausal lymph node negative breast cancer patients with a tumor size of less than 30 mm, between 1976 and 1990, to be randomized to receive adjuvant tamoxifen versus not. From the original randomized trial cohort approximately half of the patients (778 patients) had primary tumor formalin-fixed paraffin-embedded blocks available and were included in our study. No significant differences in age and period of diagnosis, type of surgery received, receptor status, tumor grade and size were observed between the treatment arms.

All tumor slides were immunostained in a central laboratory using the SP1 antibody. ER slides were scored by two independent breast cancer pathologists assessing the fraction of cancer cells for each ER intensity level (0, +1, +2 or +3) compared to established standards. The resulting distribution of ER stained tumor cells defines intra-tumor heterogeneity of ER (Rao's quadratic entropy (QE), Potts et al, Lab Invest 2012). Intra-tumor heterogeneity was categorized using the third tertile as cut-off for high heterogeneity (726 patients).

Analyses of long-term breast cancer specific survival (25 years) by intra-tumor heterogeneity of ER were performed using univariate Kaplan-Meier and multivariate Cox proportional hazard modeling adjusting for treatment arm, age and period of diagnoses, ER, progesterone receptor (PR), HER2, Ki-67, tumor grade, and tumor size. Further, a test of correlation was performed to investigate whether intra-tumor heterogeneity of ER was correlated to the percentage of ER positive cells, the H-Score or the Luminal A and B subtype (PAM50).

Results

In the univariate Kaplan-Meier analyses, a statistically significant difference in long-term survival by intra-tumor heterogeneity of ER was seen for all patients (log rank, $P=0.018$), tamoxifen treated arm (log rank, $P=0.0033$), but not untreated arm (log rank, $P=0.19$). However in the multivariate analysis, patients with high intra-tumor heterogeneity of ER in the treated arm as well as in the untreated arm had an almost two-fold increased long-term risk of fatal breast cancer disease as compared to patients with low or intermediate heterogeneity (Treated arm: HR, 2.06; 95% CI, 1.04-4.07 and Untreated arm: HR, 1.71; 95% CI, 1.01-2.87). No significant correlation of intra-tumor heterogeneity to the tested variables was seen.

Conclusions

Patients with high intra-tumor heterogeneity of ER had less benefit from tamoxifen therapy and an increased long-term risk of fatal breast cancer disease. Our findings should be clinically relevant since therapy benefit was evaluated in a randomized trial with long-term follow-up.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-04

Title: Evaluation of intra-tumor heterogeneity, test reproducibility and their impact in breast cancer samples assessed by Prosigna™: Results from a decision impact prospective study and a matched case-control study

Rouzier R, Bonneau C, Cayre A, Hequet D, Gentien D, Bonhomme A, Mouret-Reynier M-A, Dubot C, Cottu P, Roulot A, Morel P, Salomon A, Callens C, Guinebretiere J-M and Penault-Llorca F. Institut Curie, Saint-Cloud, France; Centre Jean Perrin, Clermont Ferrand, France and Nanostring.

Body: Background: Recent molecular biology technologies reveals insight into tumor heterogeneity but quantification and impact on reproducibility of tests is not well known. The objective of this study was to assess the extent to which tumor heterogeneity may affect the prognosis of patients assessed by Prosigna™ (PAM50) gene signature assay compared to test reproducibility. Methods: Reproducibility was measured by testing replicate tissue sections from 186 FFPE breast tumor blocks across 2 sites (Institut Curie, Centre Jean Perrin) following independent pathology review at each site. Consecutive slides came from blocks of patients included in the Decision Impact prospective study which examined whether the Prosigna™ test influences adjuvant treatment decision (Clinical trial information: NCT02395575). To evaluate heterogeneity and its impact in terms of outcome, we selected among T1N0 patients treated in Institut Curie between 2003 and 2008, 32 patients who did recur and 28 matched control group who did not (2 'controls' recurred during the study). Analyses were performed on two parts of each tumor. NanoString's Prosigna™ outputs (risk of recurrence (ROR) score, 10 year probability of distant recurrence, risk category, and intrinsic subtype (Luminal A/B, HER2-enriched, Basal-like)) were measured and compared to evaluate heterogeneity (defined as difference in terms of subtype and/or risk category between the two parts) and reproducibility. Correlation between heterogeneity and outcome was performed. Impact was assessed by tumor board analysis.

Results: Pearson correlation coefficients for ROR score and probability of distant recurrence predicted were .95 and .97, respectively in the reproducibility study and .82 and .86, in the tumor heterogeneity study. The measured standard deviation (SD) was 5.4 and 8.1 ROR units corresponding to 1.6% and 2.8% in terms of risk of distant metastasis free survival within the reproducibility study and the tumor heterogeneity study, respectively. Kappa coefficients for intrinsic subtype and risk category agreement were 0.88 and 0.87 in the reproducibility study, and 0.67 and 0.58 in the tumor heterogeneity study. Tumor board analysis of discordant cases showed that the impact, in terms of decision of chemotherapy administration, concerns 3% of patients because of reproducibility and 8% because of tumor heterogeneity, comparing favorably with the discordance between Prosigna™ and immunohistochemistry (27%). Probability of distant recurrence was higher in the cases (15%) compared to control (9%) ($p=.001$) in the tumor heterogeneity study confirming the performance of the Prosigna™ test.

Conclusion: We validated in the prospective Decision Impact study the analytical performance of NanoString's Prosigna™ assay across multiple clinical testing laboratories. We showed in these two studies that tumor heterogeneity has more impact than reproducibility performance. The clinical impact on the decision making based on tumor heterogeneity is however limited, since it does not correlate to outcomes, whereas the Prosigna™ ROR score has been shown to correlate very well to outcomes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-05

Title: Prediction of survival after neoadjuvant chemotherapy for breast cancer by evaluation of tumor-infiltrating lymphocytes (TILs) and residual cancer burden (RCB)

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Body: Background: The tumor immune environment not only modulates the effects of immunotherapy, but also the effects of other anticancer drugs and treatment outcomes. Thus, the importance of inhibiting and improving the tumor immune microenvironment is now recognized. These immune responses can be evaluated with tumor-infiltrating lymphocytes (TILs), which has frequently been verified clinically. On the other hand, residual cancer burden (RCB) evaluation has been shown to be a useful predictor of survival after neoadjuvant chemotherapy (NAC). In this study, RCB and TILs evaluations were combined to produce an indicator that we have termed “RCB-TILs”, and its clinical application to NAC for breast cancer was verified by subtype-stratified analysis.

Materials and Methods: A total of 177 patients with resectable early-stage breast cancer were treated with NAC. The correlation between TILs evaluated according to the standard method, and prognosis, including the efficacy of NAC, was investigated retrospectively. The RCB was calculated using the Residual Cancer Burden Calculator on the website of the MD Anderson Cancer Center. The RCB and TILs evaluations were combined to create the “RCB-TILs”. Patients who were RCB-positive and had high TILs were considered RCB-TILs-positive, and all other combinations were RCB-TILs-negative.

Results: Univariable analysis of patients with high TILs found that this contributed significantly to prolonging DFS in all patients ($p = 0.022$, hazard ratio = 0.420), TNBC patients ($p = 0.004$, hazard ratio = 0.177), and HER2BC patients ($p = 0.026$, hazard ratio = 0.123). For HRBC patients, however, high TILs did not contribute to survival ($p = 0.990$, hazard ratio = 0.992). Being RCB-TILs-positive, however, contributed significantly to prolonging DFS in all patients ($p < 0.001$, hazard ratio = 0.181), TNBC patients ($p < 0.001$, hazard ratio = 0.099), HER2BC patients ($p = 0.026$, hazard ratio = 0.123), and HRBC patients ($p = 0.039$, hazard ratio = 0.258). On multivariable analysis, being RCB-TILs-positive was an independent factor for recurrence after NAC in all patients ($p < 0.001$, hazard ratio = 0.048), TNBC patients ($p = 0.018$, hazard ratio = 0.041), HER2BC patients ($p = 0.036$, hazard ratio = 0.134), and HRBC patients ($p = 0.002$, hazard ratio = 0.081).

Conclusion: The results of the present study suggest that RCB-TILs is a significant predictor for breast cancer recurrence after NAC and may be a more sensitive indicator than TILs alone.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-06

Title: Analytical and clinical validation of a fully automated tissue-based quantitative assay (MetaSite *Breas^f*TM) to detect the likelihood of distant metastasis in hormone receptor (HR)-positive, HER2-negative early stage breast cancer (ESBC)

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Body: Background: MetaSite *Breas^f*TM is a validated assay to predict risk of distant breast cancer metastasis in patients with HR+/HER2- ESBC. The assay measures the number of MetaSites defined as tumor microanatomic structures composed of MENA protein expressing tumor cells in contact with CD31+ endothelial cells and CD68+ macrophages. Previous studies have demonstrated that an increased number of these microanatomic structures is associated with distant metastasis (DM) in HR+/HER2- ESBC independent of clinicopathologic features. Analytical validation of MetaSite *Breas^f*TM demonstrated precision of 97-99% (repeat image analysis of the same slide) and performance of 91-96% (staining and image analysis of serial tumor sections). We sought to further understand the importance of the MetaSite in predicting distant breast cancer metastasis utilizing a fully automated prognostic assay in an independent large patient cohort.

Methods: We conducted a nested case-control study within a cohort of 3,760 patients diagnosed between 1980 and 2000 with invasive breast cancer from the Kaiser Permanente Northwest health care system. Cases (n=259) were women who developed a subsequent distant metastasis; controls, selected using incidence density sampling, were matched closely to cases (1:1) on age at and calendar year of primary diagnosis. Of the 481 patient tumor samples evaluated in this study, 57% were HR+/HER2-, 19% were triple negative (TN), and 15% were HER2+ disease. Multivariate models were adjusted for clinical factors including: lymph node status, tumor size, tumor grade, and HRT; as well as matching variables: age and year of diagnosis. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic regression.

Results: In the HR+/HER2- group, MetaSite Score (MS) ranged from 0-357 and the mean was 44.6. MS was a significant predictor of DM (P=0.039) in patients with HR+/HER2- disease. Cut-points based on tertiles of MS in all 259 controls defined intermediate (13-41) and high (>41) risk groups that were significantly associated with risk of DM versus the low risk group (OR=2.24; 95%CI=1.23-4.13, P=0.009) and (OR=2.94; 95%CI=1.62-5.41, P=0.0005), respectively. Univariate estimates of absolute risk of DM with cutoffs based on 90% sensitivity and specificity were 9.4% for the low risk group (MS<7), 14.1% for the intermediate (MS=7-91), and 23.4% for the high (MS>91). When adjusted for clinical factors, estimates of absolute risk of DM were 6.6%, 14.1%, and 33.0% for the low, intermediate, and high risk groups, respectively. A binary cut-point for the high risk group was determined (MS>14) and was significant with a 2-fold higher risk of DM versus the low risk group and adjusted for clinical covariates (P=0.036). MS was not positively associated with DM in TN or HER2+ disease.

Conclusions: MetaSite *Breas^f*TM significantly predicted the risk of distant breast cancer metastasis in ESBC patients with HR+/HER2-disease, independent of classical clinicopathologic features.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-07

Title: Comparison of the Xpert breast cancer stratifier mRNA assay with central ER, PR, HER2, and Ki67 immunohistochemistry (IHC) for rapid biomarker analysis in developing countries

Brock JE E, Milner DA A, Ho K, Natalie W, Victor C, Annaliza R, Teresa B, Kathryn G-F, Edwin LW W, Jodi W, Wendy W and Michael B. Brigham and Women's Hospital, Boston, MA and Cepheid, Sunnyvale, CA.

Body: Breast cancer care in the developing world is limited by access to quality ER and HER2 IHC diagnostic assays needed to justify hormone and HER2 therapeutics. Shipping pathology specimens to a central testing site often out of country delays therapy and is costly. The Xpert Breast Cancer Stratifier assay makes quantitative measurements of *ESR1*, *PGR*, *ERBB2*, and *MKi67* mRNAs from FFPE specimens in <2 hours on an easy-to-use automated diagnostic platform, the GeneXpert (GX). 10,000 GX machines are currently in use in 182 countries offering the possibility of a point-of-care solution. We compared concordance in tumor samples between IHC and mRNA intending to challenge the limits of the GX mRNA assay.

83 breast tumor samples were chosen including those with low cellularity, small volume disease, unusual subtypes, ER- tumors with surrounding benign epithelium, and low level HER2+ tumors. mRNA, IHC and FISH assays were performed. Slides were tested following macrodissection of invasive carcinoma and as non-macrodissected whole sections. GX measurements for Ki67 were compared with mitotic rate as an alternative to Ki67 IHC.

Overall percent agreement following macrodissection was 95% for ER, 89% for HER2, 76% for PR, and 80% for Ki67 (>20% positive cut), and using whole section, 99% for ER, 80% for PR, 92% for HER2, and 73% for Ki67. Concordance was 92% for both macrodissection and whole section using mitotic rate to assess proliferation. Ignoring HER2 2+ calls which represented low level amplified tumors by FISH, the concordance rates were 95% for macrodissection and 99% for whole section. Discordance when testing long-term stored 4µm sections was resolved in a number of cases by using a fresh cut from the FFPE block. Half the ER discrepancies were in very small volume tumors $\leq 25\text{mm}^2$ and 75% were classified as ER-ve by IHC, and positive by Stratifier. 80% of ER IHC- cases were appropriately identified as ER- by the Stratifier in the presence of benign breast epithelium. HER2+ DCIS adjacent to HER2- invasive tumor resulted in a discrepant HER2 mRNA result even with macrodissection. No ER or HER2 discrepancies occurred in low cellularity tumors ($\leq 30\%$ cellularity) nor in lobular and mucinous subtypes.

In a study intended to challenge an mRNA breast biomarker assay, concordance between mRNA results and IHC was high for ER and HER2, the two most important prognostic markers needed for therapeutic decision making. Use of whole sections rather than tumor macrodissection did not decrease concordance. Discrepant ER cases were more prevalent when analyzing low volumes of tumor and in this setting were seen in ER IHC- tumors surrounded by ER+ normal epithelium, or with weak IHC expression, highlighting predictable limitations of the assay. Concordance was better between Ki67 mRNA and mitotic rate than with IHC. Re-test data suggested that a fresh cut of the FFPE block yields the best results by GX, perhaps due to mRNA degradation in stored 4µm sections. The Xpert Breast Cancer Stratifier may provide a rapid, cost-effective solution to the problem of obtaining accurate diagnostic results at the point-of-care in low resource settings, and deserves further evaluation in developing countries.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-08

Title: Impact of treatment history on prognostic ability of breast cancer index (BCI): Subset analysis from a validation study of patients with hormone receptor-positive (HR+) breast cancer with 1-3 positive nodes

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Body: Background: A new BCI model integrating tumor size and grade (BCIN+) was specifically developed and validated for prediction of risk of overall (0-15y) and late (5-15y) distant recurrence (DR) in HR+ women with 1-3 positive nodes (N1). The objective of this study was to evaluate the impact of treatment history on the prognostic performance of BCIN+ in a large clinical validation cohort of pre- and post-menopausal HR+, N1 patients.

Methods: The validation cohort was comprised of 402 HR+, N1 patients diagnosed at Massachusetts General Hospital between 1993-2007 with at least 5y of follow-up. BCIN+ risk scores were determined and patients stratified into low or high risk categories using a pre-specified cut-point blinded to clinical outcome. Kaplan-Meier estimates of overall (0-15y) and late (5-15y) DR were estimated and the difference was evaluated by log-rank test. Treatment-specific subsets were analyzed based on adjuvant endocrine (tamoxifen [TAM] only vs any history of aromatase inhibitors [AI]), and adjuvant chemotherapy treatment history.

Results: Mean age of patients was 53y. 99% were ER+, 91% PR+, and 13% HER2+. The majority of tumors were T1 (62%) or T2 (35%). Adjuvant endocrine treatment included TAM only for 191 (48%) patients and either AI only or a sequence of TAM and an AI in 211 (52%) patients. Most patients received chemotherapy (n=324; 81%). BCIN+ classified 20% and 80% as low and high risk, respectively.

In patients treated with TAM only, BCIN+ low and high risk had significantly different 15y rates of DR (95% CI) of 4.0% (0.0-11.4%) vs 41.7% (33.0-49.3%), respectively ($p=0.0005$). For patients disease-free at year 5, rates of late DR (5-15y) were 4.0% (0.0-11.5%) vs 20.0% (11.4-27.8%), respectively ($p=0.04$). In patients treated with an AI, BCIN+ low and high risk had significantly different 15y rates of DR of 0% (0.0-0.0%) vs 15.0% (8.1-21.5%), respectively ($p=0.006$). For patients disease-free at year 5, rates of late DR were 0.0% (0.0-0.0%) vs 12.2% (5.6-18.3%), respectively ($p=0.02$). There was no statistically significant difference in the prognostic performance of BCIN+ between patients treated with TAM only versus those with treatment including any history of AI (interaction $p=0.99$).

In the subset of patients treated with chemotherapy, BCIN+ classified 19% and 81% of patients as low and high risk with significantly different 15y rates of DR of 1.7% (0.0-4.9%) vs 30.9% (24.4-36.8%), respectively ($p<0.0001$). For patients disease-free at year 5, rates of late DR were 1.7% (0.0-4.9%) and 16.3% (10.2-21.9%), respectively ($p=0.006$).

Conclusions: In this subset analysis from a validation study of N1 patients, BCIN+ identified a significant proportion with a significantly low risk of late DR. This study confirms the ability of BCIN+ to identify a subset of patients with significantly low risk of recurrence across adjuvant endocrine and chemotherapy treatment backgrounds. BCIN+ may provide additional prognostic information to facilitate selection of N+ patients for extended endocrine treatment, wherein patients identified as BCIN+ low may be considered adequately treated with adjuvant therapy alone.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-09

Title: Programmed death 1 (PD-1) and PD-1 ligand (PD-L1) distribution in triple negative breast cancer (TNBC)

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Body: Background: Recent data indicate a promising response to immune checkpoint inhibition in patients with metastatic TNBC. Ample research showed that PD-L1, a PD-1 ligand, is expressed in multiple tumor types, including TNBC, and may be a predictor of response to PD-1/PD-L1 blockade. Quantification of the stromal composition, particularly PD-1 and PD-L1 expression, continues to be controversial in its relationship to immune checkpoint inhibition in several cancer types, and it remains unclear whether PD-L1 expression is necessary to predict response. Here, we aimed to determine the distribution of PD-1 and PD-L1 in a large set of centrally ascertained specimens of TNBC.

Methods: The study cohort consisted of 993 tumor samples (both primary and metastatic TNBC) analyzed for either PD-1 or PD-L1 expression in one laboratory (Caris Life Sciences; Phoenix, AZ). Estrogen receptor and progesterone receptor status was assessed by immunohistochemistry (IHC). HER2/Neu expression or amplification was assessed by either IHC or in-situ hybridization. PD-1 and PD-L1 expression were confirmed using IHC with validated antibodies. For PD-L1, clone SP142 (Roche Diagnostics) was utilized and a sample was considered positive if there was $\geq 5\%$ membranous staining of tumor cells. For PD-1, clone EH21.1 (BD Biosciences) was used. Tumor infiltrating lymphocytes (TILs) expressing PD-1 were counted and a sample was considered positive if there was at least one PD-1 positive TIL per 40x microscopic field.

Results: The median age in this cohort was 56 years (range: 22 – 88). A total of 363 TNBC specimens were tested for PD-1 via IHC. One hundred fifty eight (158; 43.5%) were negative for PD-1 expression. Two hundred five (205; 56.5%) were positive for PD-1. Of those that were PD-1 positive, 116 (56.6%), were in samples from a primary site (breast) and 89 (43.4%) in samples from a metastatic site. A total of 630 TNBC specimens were tested for PD-L1 via IHC. Five hundred seventy four (574; 91.1%) were negative for PD-L1. Fifty-six (56; 8.9%) were positive for PD-L1. Of those that were PD-L1 positive, were equally distributed between primary site and metastatic sites (28/324, and 28/306, respectively).

Conclusion: In this retrospective analysis, we describe, to the best of our knowledge, the distribution of PD-1 and PD-L1 expression in one of the largest datasets reported in TNBC. Unlike prior reports showing a high PDL-1 expression in excess of 50% in TNBC, this analysis show a low distribution of PD-L1 positivity. Our cohort represents a biased sample as those were unselected patients with recurrent breast cancer. Additionally, other factors can be implicated, including a change in the antibody used. These findings call for future standardization of the PD-L1 assay, particularly if further exploration showed PD-L1 to be a predictive or prognostic biomarker in mTNBC, particularly in relationship to therapy with immune checkpoint blockade.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-10

Title: UCBG 2-14: A prospective multicenter non-randomized trial evaluating the effect of EndoPredict® (EPclin®) clinico-genomic test on treatment decision making among patients with intermediate clinical risk

Penault-Llorca F, Kwiatkowski F, Grenier J, Levy C, Leheurteur M, Uwer L, Derbel O, Le Rol A, Jacquin J-P, Jouannaud C, Quenel-Tueux N, Girre V, Foa C, Guardiola E, Lortholary A, Catala S, Lemonnier J and Delalogue S. Centre Jean Perrin, Clermont Ferrand, France; Institut Sainte Catherine, Avignon, France; Centre François Baclesse, Caen, France; Centre Henri Becquerel, Rouen, France; Institut de Cancérologie de Lorraine, Vandoeuvre les Nancy, France; Hôpital Privé Jean Mermoz, Lyon, France; Centre Hospitalier Intercommunal, Quimper, France; Institut de Cancérologie Lucien Newirth, Saint Priest en Jarez, France; Institut Jean Godinot, Reims, France; Institut Bergonié, Bordeaux, France; Centre Hospitalier Départemental de Vendée, La Roche sur Yon, France; Hôpital Saint Joseph, Marseille, France; Centre Hospitalier de la Dracénie, Draguignan, France; Centre Catherine de Sienne, Nantes, France; Centre Catalan d'Oncologie, Perpignan, France; R&D Unicancer, Paris, France and Gustave Roussy, Villejuif, France.

Body: Background: Genomic tests can identify ER-positive Her2-negative localized breast cancer (BC) patients (pts) who may not drive any benefit from adjuvant chemotherapy (CT). Several genomic tests have reached a high level of analytical and clinical validity, as well as clinical utility in such situation. Recent results suggest that the safe de-escalation of adjuvant chemotherapy may be most beneficial in pts with clinical high or indeterminate risk, as assessed by classical variables or online tools, through the use of a genomic test. The clinical risk though remains quite uncertain with variable definition and grey zones. The present study aimed at determining if EPclin clinico-genomic test had a significant impact on treatment decision making among pts with predefined intermediate /borderline clinical risk.

Patients and methods: Women were eligible for the present study if they had complete surgical removal of a localized ER+ Her2- pN0 or pN1mi BC, and were considered by the multidisciplinary team meeting (MTM) of the center as being in a "grey zone" of uncertain CT benefit based on classical clinic-pathological assessment. The MTM1 proposed a decision (chemo/no chemo). After informed consent, an EPclin signature classified the tumor as low risk (EPclin Score < 3.3, no chemo advised) or high risk (≥ 3.3 , a theoretical indication for adjuvant chemo). Primary end point was the proportion of change between initial adjuvant CT decision at MTM1 and final administration of CT (yes/no). A 5 steps Fleming design was planned, considering that a change rate of 15% or less was not acceptable (low clinical utility). A one-step design was used (unilateral $\alpha = 2.5\%$ and $\beta = 1\%$).

Results: 203 pts were included, of whom 198 are evaluable for the main end point. 74% of the tumors were grade 2, 72% T1, 25% T2, 3% T3; 16% were pN1mi and 84% pN0. Median age was 57. EpClin® was low risk in 67% and high risk in 33% of the cases.

The global rate of decision change between MTM1 and final administration of CT was 70/198 (35.4% IC-95% = [28.7-42.1]), with 55 (27.7%) decreases and 15 (7.6%) increases in CT indication. 27% instead of 47% of all pts finally received CT (43% decrease in CT prescription). In the multivariate analysis, the factors associated to decrease for less CT were EPclin score (OR 0.11 [0.03-0.35]), proliferation (OR 1.08 [1.03-1.13]) and higher grades (OR 5.22 [1.04-26.08]); while EPclin score was positively (OR 76.6 [7.11-824.8]), but higher grades inversely (OR 0.06 [0-0.86]) associated to an increase in CT administration.

Of note, the change in final decision occurred after the MTM2 and after report of the results and discussion with the patient in 9 of the 70 cases (12% of changes; 4.5% of the whole population)(8 decreases and 1 increase in CT administration).

Conclusion: Adenom met its primary objective, with 35% of intermediate clinico-pathological risk pts getting significant therapeutic changes upon the receipt of an EPclin gene expression profile. 43% of these "intermediate risk" pts planned for CT avoided it. 12% of the changes were discrepant with the test's results and occurred after discussion with the patient.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-11

Title: miR363-3p mediates maintenance and resistance of breast cancer stem cells (BCSC)

Renaud S, Fiche M, Stravodimou A, Scabia V, Dormoy VM, Galmiche Rindisbacher M, Brisken C, Mermoud N and Zaman K. Laboratory of Molecular Biotechnology, University of Lausanne, Lausanne, Switzerland; University Hospital CHUV, Lausanne, Switzerland; Breast Center, University Hospital CHUV, Lausanne, Switzerland; Ecole Polytechnique Fédérale (EPFL), Switzerland and CHU Maison Blanche, University of Reims Champagne-Ardenne (URCA), France.

Body: Background

BCSC are considered to be involved in the recurrence of breast cancer and its resistance to the systemic therapies. Their detection and targeting remain challenging.

Patients and methods:

The study was conducted in vitro, in xenografted immunocompromised mice and samples of 38 patients with early stage BC having biopsies and blood samples before and after anthracycline + taxane-based neoadjuvant chemotherapy (NAC). All patients gave written informed consent before inclusion. 1) MCF7 cells grown as mammospheres (MS) for BCSC enrichment were treated with 5FU or paclitaxel (Pac) to select chemo-resistant BCSC. miRNA microarray was performed to identify specific miRNAs for chemo-resistant BCSC. The results were compared to miRNAs found in immortalized non-tumorigenic MCF10A cells to exclude miRNAs related to normal stem cells. 2) The correlation between the most highly expressed miRNA and the BCSC was confirmed by RT-qPCR in ALDH+ and ALDH- cells sorted from MCF7 and MDA-MB-231 cells by flow cytometry. 3) The impact of the miRNA on MS and colony development was assessed by up- and down-regulating its expression. MCF7 cells transfected with ectopic expression of miRNA, anti-miRNA or miRNA control were grown in MS before being injected in mice using a mouse INtraDuctal xenograft Model (MIND). In vivo tumor growth was assessed by luciferase imaging, then measured and quantified with human GAPDH ex vivo at 6 weeks. 4) The miRNA was quantified by RT-qPCR in tumor samples and sera of the patients before and after treatment, and its levels were correlated with pathological complete response and patients' outcomes.

Results:

379 miRNAs out of 2006 were altered in chemo-resistant versus untreated MCF7 MS. Thirteen were specific for 5FU and 5 for Pac. Three were common for both drugs. Of these, miR-363-3p was overexpressed specifically in BCSC-enriched chemo-resistant MCF7 cells and all the other tested BC cell lines, but not in non-tumorigenic MCF10A cells. Compared to adherent MCF7 cells, miR-363-3p was 12-, 60-, and 10-folds more expressed in BCSC-enriched MS treated with 5FU, Pac, or without treatment, respectively. miR-363-3p was 20- and 100-folds higher in ALDH+ compared to ALDH- in MCF7 and MDA-MB-231 cells. Anti-miR-363-3p reduced MS size and decreased their number 50%. A significant decrease of the number of colonies was also observed in soft agar. Consistently, miR-363-3p downregulation decreased tumor growth and metastasis by MCF7 cells transplanted in mice. In patients' sera with lower baseline level (n=15), miR-363-3p appeared decreased upon NAC. Patients with high miR-363-3p serum levels (n=22) had more risk to maintain higher level after chemotherapy. Triple-negative and HER2+ BC were more frequent in this second group. No significant difference was observed in term of pCR between the 2 groups. However 3 patients relapsed with distant metastases and all were in the second group with high baseline level and no decrease after NAC.

Conclusions:

miR363-3p appeared to be a mediator of chemo-resistant BCSC. Its measurement in the serum of BC patients may predict resistance to neo-/adjuvant chemotherapy and higher risk of distant recurrence. Further investigations are warranted to confirm its role as biomarker and potential therapeutic target against BCSC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-12

Title: The ASCO-recommended prognostic factors uPA/PAI-1 in a multicenter cohort study (PiA)

Vetter M, Hartung C, Hanf V, Lantzsch T, Uleer C, Peschel S, John J, Buchmann J, Bürrig K-F, Weigert E, Thomssen C and Kantelhardt EJ Johanna. Martin-Luther-University, Halle (Saale), Germany; Klinikum Fürth, Fürth, Germany; St. Elisabeth St. Barbara, Halle (Saale), Germany; Praxis Uleer, Hildesheim, Germany; Klinikum St. Bernward, Hildesheim, Germany; Klinikum Hildesheim, Hildesheim, Germany; Institut of Pathology, Krankenhaus Martha-Maria, Halle (Saale), Germany; Institut of Pathology Hildesheim, Hildesheim, Germany and Institut of Pathology, Klinikum Fürth, Fürth, Germany.

Body: Introduction

The PiA-study (Prognostic assessment in routine Application, NCT 01592825) was designed as a representative cohort of breast cancer patients to estimate the proportions of traditional and modern prognostic factors. The ASCO-recommended biomarkers uPA (urokinase-type plasminogen activator) and its inhibitor PAI-1 were used for biological risk assessment particularly for intermediate risk breast cancer patients and disease-free survival of the patients after 5 yrs of follow-up (F/U) was calculated.

Material & Methods

Between 2009 and 2011, 1,074 non-metastasized, primarily operated breast cancer patients from six centers in Germany were included. From 815 patients, fresh frozen tissue was obtained and processed for central testing uPA/PAI-1 by ELISA (FEMTELLE®, Sekisui Diagnostics GmbH). Low uPA/PAI-1 status is defined by uPA and PAI-1 concentrations below the published cut-offs, high status means one or both were higher than the corresponding cut-offs. Tumor characteristics were based on local pathology. The centers had to follow the national guidelines. In low-risk patients, adjuvant chemotherapy was spared. The median F/U is 56 months (range 0-78).

Results

In the total cohort of 1,074 patients, 166 had G1- and 237 had G3-tumors. Of the 671 patients with a G2-tumor, the following were allocated to the high-risk group: node-positive (n=371), younger than 35 yrs (n=17), and triple-negative (TN) or HER2-positive (n=118). For 253 tumors of the remaining 355 patients with an intermediate risk of recurrence (pN0, G2, HR positive, HER2-negative, ≥35 yrs), uPA/PAI-1 status was available. 126 (49.8%) were allocated to the low-risk group, one patient had a recurrence. At 5 yrs, in the total cohort 90.6% (95% CI, 89.5-91.7) of the patients were free of invasive disease. Of 114 HER2-positive tumors, 94 (82.4 %) had a high uPA/PAI-1 status, only one of the 38 HR negative/HER2 positive tumors had a low uPA/PAI-1 status. In the TN group, the majority of tumors had a high uPA/PAI-1 status (66 of 81; 81.5 %). In 30 patients lymph nodes were involved, 18.5% (n=15) had a low uPA/PAI-1 status, one event was detected. In N pos. patients with an high uPA/PAI-1 6 events were observed.

Conclusion

Testing for uPA/PAI-1 in the daily routine is feasible, fresh frozen tissue has been prepared from 76% of the tumors of the recruited patients, 37% of them had a low risk status. Using uPA/PAI-1, about half of the node-negative patients with an intermediate risk of recurrence were allocated to a group with an extremely low risk of recurrence and thus chemotherapy could be spared. Also in node-positive disease, uPA/PAI-1 has a prognostic impact.

Tab 1: Proportion of the subgroups according to IHC, grading and uPA/PAI-1-status

Tumor type	total n=815 (100%)	low uPA/PAI-1 status n=304 (37%)	high uPA/PAI-1 status n=511 (63%)
<i>Luminal A-like tumors:</i> HRpos., HER2neg., G1, G2	515 (63.2%)	240 (78.9%)	275 (53.9%)
<i>Luminal B/HER2-negative-like tumors:</i> HRpos., HER2neg., G3	104 (12.8%)	29 (9.5%)	75 (14.7%)
<i>Luminal B/HER2-positive-like tumors:</i> HR pos., HER2 pos., all grades	77 (9.4%)	19 (6.2%)	58 (11.4%)
	38 (4.7%)	1 (0.3%)	37 (7.3%)

<i>HER2-positive (nonluminal) - like tumors:</i> HRneg., HER2pos., all grades			
<i>TN tumors:</i> HRneg., HER2neg., all grades	81 (9.9%)	15 (4.9%)	66 (12.9%)

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-13

Title: Negative progesterone receptor is associate early breast cancer relapse, even among good prognosis tumors

Winner M, Rosman M, Mylander C, Jackson RS S, Pozo ME E, Wolff AC C, Tafra L and Umbricht CB B. Johns Hopkins University School of Medicine, Baltimore, MD and The Breast Center, Anne Arundel Medical Center, Annapolis, MD.

Body: Background/objective: A minority of estrogen-receptor (ER) positive breast cancers lack progesterone receptor (PR) expression, but little is known of the clinical meaning of PR negativity (PR-). In the present study we sought to clarify the association between PR- and outcomes of ER+, human-epidermal growth factor (HER2)-negative breast cancers using a large, single institution database.

Methods: We retrospectively analyzed consecutive, non-metastatic, unilateral HER2- invasive breast cancers diagnosed between 2000 and 2011. Records were reviewed for age at diagnosis, disease stage, tumor features, and histologically confirmed recurrence. ER+ and PR+ status was defined as $\geq 1\%$ immunoreactive cells. We used Kaplan-Meier curves to determine the association between PR- and early (≤ 5 years) and late (>5 years) disease recurrence, defined as locoregional or distant breast cancer relapse >6 months after diagnosis.

Results: We identified 1,933 patients with TN (n=337) or ER+/HER2- (n=1,596) breast cancer. Patients with ER+/PR- (n=107) vs. ER+/PR+ (n=1,489) tumors did not differ in age or disease stage at diagnosis; however, PR- tumors were more frequently high grade (37.9% vs. 17.8%, $p < 0.001$), with higher median Ki67 indices (20.0% vs. 10.0%, $p < 0.001$). Median ER expression was also lower in PR- as compared to PR+ tumors (80.0% vs. 90.0%, $p < 0.001$).

Over a median follow-up of 84 months, there were 119 early and 54 late locoregional or distant breast cancer relapses. Negative PR was strongly associated with early relapse, with PR- tumors demonstrating a 2.1-fold higher hazard of relapse in the first 5 years as compared to PR+ tumors (95% CI 1.0-4.2)

Hazards of early (<5 years) breast cancer relapse by hormone status. Shown are univariable Cox proportional hazard ratios and 95% confidence intervals among all tumors, and in subsets defined by %ER, node status, Ki67, and grade.

	All tumors n=1,933	High ER (80-100%) n=1,383
TN	3.9 (2.6-5.6)*	--
PR 0%	2.1 (1.0-4.2)*	1.7 (0.6-4.6)
PR 1-100%	Reference	Reference
	Node-negative n=1,299	Node-positive n=634
TN	4.3 (2.5-7.5)*	3.6 (2.1-6.0)*
PR 0%	2.7 (1.0-7.0)*	1.6 (0.6-4.5)
PR 1-100%	Reference	Reference
	Ki67 <14% n=768	Ki67 $\geq 14\%$ n=997
TN	**	2.4 (1.5-3.8)*
PR 0%	4.1 (1.2-14.1)*	1.6 (0.7-3.8)
PR 1-100%	Reference	Reference
	Grade 1/2 n=1,337	Grade 3 n=564
TN	3.4 (1.4-7.9)*	1.9 (1.2-3.3)*
PR 0%	2.0 (0.7-5.7)	1.2 (0.4-3.5)
PR 1-100%	Reference	Reference

* $p < 0.05$; **too few subjects/events for analysis

. Negative PR remained significantly associated with a higher hazard of early relapse even in node-negative (HR 2.7, 95%CI 1.0-7.0) and low-proliferating tumors (Ki67<14%, HR 4.1, 95%CI 1.2-14.1). There was no significant association between PR-

and late breast cancer relapse (HR 0.7, 95%CI 0.2-2.9).

Conclusions: Compared to ER+/PR+ breast cancers, ER+/PR- breast cancers have a significantly greater risk of early recurrence, similar to triple-negative cancers. These results suggest that negative PR expression is importantly and independently associated with early breast cancer prognosis, and may be an indicator of unique tumor biology.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-14

Title: Prognostic impact of genomic risk stratification with breast cancer index in patients with clinically low risk, hormone receptor-positive, node-negative, T1 breast cancer

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Body: Background: Tumor size and nodal status are prognostic for risk of both early and late disease recurrence in patients with early stage, HR+ breast cancer, and are incorporated into both adjuvant chemotherapy and extended endocrine therapy treatment decisions. In a recent EBCTCG meta-analysis of over 46,000 patients [Pan H, et al. J Clin Oncol 34, 2016 (suppl; abstr 505)], risk of late distant recurrence was assessed in patient subsets based on nodal status and tumor size. Patients with T1N0 disease who were treated with 5 years endocrine therapy had a good overall prognosis, with 4%, 9%, and 14% risk of distant recurrence from years 5-10, 5-15, and 5-20, respectively. Breast Cancer Index (BCI) has been validated as prognostic biomarker for risk of both early and late distant recurrence in multiple randomized trial cohorts. The aim of this analysis was to assess distant recurrence (DR) risk stratification with BCI in patients with clinically low-risk T1N0 tumors.

Methods: Primary tumor samples from the subset of patients with T1N0 disease from 2 independent validation cohorts of HR+ breast cancer patients were examined [Stockholm randomized controlled trial (N=259) and a retrospective multi-institutional cohort (N=237)]. Patients in the Stockholm RCT cohort were treated with adjuvant tamoxifen only; patients in the multi-institutional cohort were treated with adjuvant tamoxifen +/- chemotherapy (20.3%). No patients received extended endocrine therapy. Kaplan-Meier analysis was used to assess the risk of DR within distinct BCI risk groups. Time dependent analysis was performed by combining BCI Low and Intermediate risk groups for risk of early recurrence (0-5y), and BCI Intermediate and High risk groups for risk of late recurrence (>5y).

Results: In the Stockholm cohort, BCI identified 13% of T1N0 patients as high risk for relapse within the first 5y, and these patients had a significantly reduced distant recurrence-free survival (DRFS, 85.3%) compared to BCI Low Risk patients (97.7%; P=0.0004). In patients disease-free at year 5, BCI identified 32% of patients as high risk for late recurrence; these patients had significantly lower DRFS (86.7%) between years 5-15 compared to BCI low risk patients (95.4%; P=0.0263). In the multi-institutional cohort, 22% of T1N0 patients were identified by BCI as high risk for relapse within the first 5y, and these patients had a significantly reduced DRFS (77.3%) compared to BCI low risk patients (96.2%; P<0.0001). In patients disease-free at year 5, 36% of patients were identified by BCI as high risk for late recurrence, with significantly lower DRFS (89.6%) between years 5-10 compared to BCI Low Risk patients (98.4%; P=0.008).

Conclusions: HR+ Patients with favorable clinical features (T1N0) have a good overall prognosis. However, results of this study demonstrated that adding molecular resolution on tumor biology with BCI identified a significant subset of women with higher risk of both early and late distant recurrence; findings support consideration of genomic classification in T1N0 patients to identify additional candidates for adjuvant chemotherapy and/or extended endocrine therapy, respectively.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-15

Title: The lymph node ratio as an independent prognostic factor for node-positive triple-negative breast cancer

He M, Jiang Y-Z, Tang L-C, Chen Y-L, Shao Z-M and Di G-H. Fudan University Shanghai Cancer Center, Shanghai, China.

Body: Purpose: To evaluate the prognostic value of the lymph node ratio (LNR) in patients with axillary lymph node-positive triple-negative breast cancer (TNBC).

Patients and methods: Based on X-tile plots, we classified women into low-, medium-, and high-risk LNR groups. Univariate and multivariate Cox proportional hazards regression analyses for overall survival (OS), breast cancer-specific survival (BCSS) and disease-free survival (DFS) were performed. The prognostic efficacy of the LNR was investigated in the first cohort from the Surveillance, Epidemiology, and End Results (SEER) database (n=4114) and was further validated in an independent cohort from Fudan University Shanghai Cancer Center (n=417).

Results: The patients were classified into low- (≤ 0.30), medium- (0.30 to 0.70) and high-risk (> 0.70) LNR groups. Multivariate analysis revealed that the LNR was an independent predictor of OS (hazard ratio (HR) for high-risk LNR: 3.24; 95% confidence interval (CI): 2.56 to 4.09) and BCSS (HR for high-risk LNR: 3.57; 95% CI: 2.76 to 4.62) in the SEER population

Effect of Lymph Node Ratio and pN Classification on Survival Among Patients from SEER

Variable	OS		BCSS	
	HR a(95% CI)	P	HR a(95% CI)	P
Lymph node ratio				
Low, ≤ 0.30	1	-	1	-
Medium, > 0.30 and ≤ 0.70	2.05(1.63-2.59)	< 0.001	2.26(1.75-2.92)	< 0.001
High, > 0.70	3.24(2.56-4.09)	< 0.001	3.57(2.76-4.62)	< 0.001
pN				
N1	1	-	1	-
N2	1.53(1.21-1.93)	< 0.001	1.63(1.27-2.10)	0.001
N3	2.72(2.14-3.46)	< 0.001	2.97(2.29-3.85)	< 0.001

a Cox proportional hazards model. Hazard ratios are adjusted for age, race, histologic grade, tumor size, surgery type and radiotherapy.

and also for DFS (HR for high-risk LNR: 4.29; 95% CI: 2.24-8.21) in the validation population. Subgroup analysis revealed that patient classification according to the LNR could discriminate among groups of patients with different survival rates based on pN staging.

Conclusion: The LNR shows potential for use as an additional prognostic factor for TNBC patients with positive lymph node involvement. Considering the heterogeneity of TNBC, use of the LNR might allow for optimization of the pN staging system, and this ratio should be considered when making treatment decisions.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-16

Title: Establishment of molecular profiling for individual treatment decisions in early breast cancer – Clinical impact of PAM50 and PAM50 risk of recurrence score after more than 16 years follow up

Naume B, Borgen E, Falk RS Sørum, Ohnstad HO Oma, Lien TG G, Aaserud M, Sveli MAT Anh Tu, Kyte JA Amund, Kristensen V, Geitvik G, Schlichting E, Wist E, Sørli T and Russnes H. Oslo University Hospital, Oslo, Norway and University of Oslo, Oslo, Norway.

Body: Background

Molecular profiling has recently been included in recommendations for decisions on adjuvant treatment in breast cancer (BrCa). However, the use of molecular profiling has not yet been widely established in all countries. Additional studies may give important information about the clinical relevance of the tests.

Aims

The study aims to discover the long term prognostic impact of PAM50 and PAM50 ROR score on survival for early BrCa pts according to treatment, with comparison to the routine clinical and histopathological parameters.

Patients and methods

Unselected early BrCa pts (n=651) from the Oslo Micromet project (n=920) having available FFPE primary tumor tissue were included in the current study. The pts were enrolled from 1995-1998. Follow up status is available for distant disease (median FU 7 years) and BrCa death (16.0-19.7 years after study inclusion). Clinical and histopathological parameters have been collected from the hospital records. FFPE tissue sections were macrodissected, RNA isolated from the dissected tumor tissue, followed by analysis of the PAM50 gene list on the Nanostring Platform. The samples were run in research mode and the raw data was sent to Nanostring (Seattle) for determination of the PAM50 subtype and ROR score.

Results

Of the 651 included pts, 323 did not receive any adjuvant systemic treatment (pT1pN0 patients), 161 tamoxifen only, the rest chemotherapy+/-tamoxifen. Twelve preoperatively treated pts were excluded from the analyses. Of the 639 remaining pts, PAM50 molecular profiling defined 52.3% as LumA, 26.8% LumB, 10.6% HER2enriched and 10.3% Basal. Multivariate analysis showed that the PAM50 intrinsic subtypes yielded prognostic information in addition to the established clinicopathological variables (pT, Grade, pN, age HR/HER2 subgroups, systemic treatment)(BCSS: HazardR vs LumA: 2.7 (95% CI 1.7-4.1) for LumB, 3.5 (1.8-6.8) for HER2enriched, 1.8 (0.8-4.2) for Basal). For the HR+HER2- pts, the risk classification by ROR score was an independent prognostic factor (BCSS: HazardR vs low risk: 3.1 (1.2-8.1) for intermediate, 6.6 (2.5-17.1) for high risk). In univariate analysis, the PAM50 intrinsic subtype classification separated clinical outcome both for all pts, for no adjuvant treated pts (both p<0.001, log rank), for the HR+HER2- (p<0.001), HR+HER2+ (p=0.061) and HR-HER2- (p=0.015) subgroups. Among the pT1-2pN0 HR+HER2- pts with no adjuvant treatment (n=222), risk classification by ROR score categorized 52.7% of the pts as low risk with excellent prognosis (BrCa death 4.2%), 29.7% as intermediate risk (BrCa death 16.7%) and 17.6% as high risk (BrCa death 35.9%)(p<0.001, log rank). For the pT1-2pN0-1 HR+HER2- pts who received adjuvant tamoxifen only (n=102), a low and similar risk of BrCa death was observed among the low and intermediate ROR risk groups. The high risk group had poor prognosis (BrCa death 32.7%)(p<0.001). Similar results were obtained for patients classified as LumA.

Conclusions

PAM50 subtype classification and ROR score improves classification of BrCa pts into prognostic groups, allowing more precise identification of future recurrence risk and improved basis for adjuvant treatment decisions.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-17

Title: Development of a recurrence risk stratification schema to inform treatment decisions in breast cancer patients undergoing mastectomy with 1-3 positive nodes

Scanlan JM M, Ellis ED D, Kaplan HG G, Kieper DA A, Morris AD D and Atwood M. Swedish Center for Research and Innovation, Seattle, WA; Providence Health and Services, Seattle, WA and Swedish Cancer Institute, Seattle, WA.

Body: Introduction Controversies exist regarding the appropriateness of radiation therapy (XRT) in mastectomy patients who have 1 – 3 positive nodes (LN+). We sought to create a straightforward recurrence and survival prediction model derived from clinical and pathologic parameters common in the community cancer center setting at the time of surgery (SX) that may help determine the patients most likely to benefit from XRT.

Subjects In this retrospective observational study, we examined our institution's breast cancer patient registry to identify mastectomy patients with one to three LN+ at the time of diagnosis and tumor pathology results including Her-2 receptor status. Consistent with current treatment standards, Her-2 positive tumors that were not treated with Herceptin were excluded.

Methods Breast cancer registry data elements including clinical, pathology, treatment and outcomes data were extracted. Logistic multiple regressions were used to predict loco-regional recurrence (LRR), distant recurrence (DR) and total recurrence (TR) as well as breast cancer mortality (BCa mortality) and all-cause mortality (ACM) based on clinical and pathologic parameters available at SX. The parameters included in the regression model were: tumor receptor status - estrogen (ER), progesterone (PR) and HER-2, the number of nodes examined, presence or absence of lymphovascular invasion (LVI), extension of LVI, number of LN+, node positivity ratio (positive/examined), surgical margins or “tumor on ink”, (SxM); and patient age.

Results The application of these filters to our breast cancer registry yielded 935 patients with a mean follow up time of 7 years. Our sample was “modern”: 95% of our patients were diagnosed after 1999, 80% after 2004. Across multiple analyses, the four most consistent risk indicators were PR(-),LVI (+), LN+>1 and SxM (+). In our model, patients with none of the four factors were designated low-risk, those with only one factor were medium-risk, those with 2+ were high-risk (see Table 1).

Risk model Validation: All patients

	N =	LRR	DR	TR	BCa Mort.	ACM
Low Risk	216	0%	3.7%	3.7%	2.8%	8.8%
Medium Risk	491	2.4%	9.6%	11.2%	5.7%	12%
High Risk	228	4.4%	12.7%	16.7%	11.8%	18.9%
3 grp. p-value		.01	.001	.001	.001	.01

From this complete group, we extracted the relatively small number of patients (N = 137) who, due to a variety of factors, received no chemotherapy or radiation therapy and which showed stronger contrasts between risk groups (see Table 2).

Risk model Validation: No chemotherapy or XRT

	N=	LRR	DR	TR	BCa Mort.	ACM
Low Risk	51	1.9%	3.9%	3.9%	3.9%	23.5%
Medium Risk	70	8.6%	10%	18.7%	8.6%	27.1%
High Risk	16	25%	31.3%	50%	50%	68.8%
3 grp. p-value		.01	.001	.001	.001	.01

Discussion We believe this risk group concept is straightforward, feasible and clinically useful in all clinical settings. Our future work seeks to validate this concept in another independent cancer registry. In our analyses we noted that while both PR- and ER- were predictive of patient outcomes in simple correlations, PR- was more predictive in all multivariate equations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-18

Title: Correlation of breast cancer index (BCI) risk classification with tumor grade and Ki-67 in a large series of patients with early-stage, ER+ breast cancer

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Body: Background: Breast Cancer Index (BCI) is a gene expression-based test that integrates biomarker panels of both proliferation (molecular grade index, MGI), and endocrine response (HoxB13/IL17BR). BCI has been validated as a significant and independent prognostic factor both for risk of overall (0-10y) and late (>5y) distant recurrence, and is predictive of extended endocrine benefit in patients with early-stage, ER+ breast cancer. In this study, correlative analyses of risk stratification by BCI and MGI versus tumor grade or Ki67 were assessed to characterize their relationship to other markers of proliferation.

Methods: Retrospective analysis of consecutive cases (N=1359) from node negative early-stage breast cancer patients submitted for clinical testing with BCI were analyzed. Median age at diagnosis was 58 years, 57% and 6% were HER2 negative and positive, respectively, with 37% unknown status. Tumor grade and Ki67 information was abstracted from pathology reports, and was available for 1335 and 372 cases, respectively. 29%, 52% and 17% of patients had grade 1, 2 and 3 tumors, respectively, with 2% having unknown grade. Tumor sizes were 26% (≤ 1 cm), 48% ($>1 - \leq 2$ cm), 23% ($>2 - \leq 5$ cm), 2% (≥ 5 cm) and 1% were unknown. Ki67 categories were based on 10% and 20% IHC expression levels into low, intermediate and high groups. Statistical methods included Pearson correlation between BCI/MGI versus Ki67 as continuous variables & coefficient of determination derived from the analysis of variance (ANOVA) model between continuous BCI/MGI versus tumor grade. Chi-square test assessed the significance of concordance between BCI, MGI risk groups to tumor grade and Ki67 groups.

Results: As continuous variables, BCI and MGI correlated weakly with tumor grade (coefficient of determination= 0.26 and 0.22, respectively) and Ki67 ($r^2 = 0.35$ and 0.33 , respectively). Although statistically significant concordance was demonstrated between BCI/tumor grade, MGI/tumor grade, BCI/Ki67, MGI/Ki67 categories ($p < 0.0001$ for all, Tables 1 & 2), discordance between BCI versus tumor grade or Ki67 was 51% and 45%, respectively. In particular, BCI classified 4% of well differentiated tumors as high-risk and 18% of poorly differentiated tumors as low-risk. Similarly, BCI classified 4% of low Ki67 patients as high risk and 28% of high Ki67 patients as low risk.

Table 1: BCI risk classification vs tumor grade

	Grade 1	Grade 2	Grade 3
BCI Low Risk	305	365	42
BCI Intermediate Risk	69	216	56
BCI High Risk	15	130	137

Table 2: BCI risk classification vs Ki67

	Ki67 Low	Ki67 Intermediate	Ki67 High
BCI Low Risk	109	47	40
BCI Intermediate Risk	21	36	45
BCI High Risk	6	8	60

Conclusions: Data from this large retrospective analysis show that correlation of BCI and its mitogenic panel, MGI, to tumor grade and Ki67 are moderate to weak. These findings indicate that BCI and MGI are capturing distinct information related to tumor proliferative status compared with tumor grade and Ki67.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-19

Title: Impact of TP53 functional mutation type on clinical outcomes of advanced breast cancer patients

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Body: Background: Next-generation sequencing (NGS) has improved our understanding of breast cancer (BC) biology. Somatic TP53 mutations (TP53m) are present in 30% of BC, and are particularly common in triple negative (TN) tumors. Although multiple studies have indicated poor prognosis in BC patients (pts) with TP53m, there is still uncertainty regarding its correlation with clinical outcomes, which may be influenced by other molecular, histological and clinical factors. Our aim was to investigate the functional effect of TP53m in advanced BC pts and evaluate associations with clinical outcomes in different BC subtypes.

Methods: Advanced BC pts enrolled in an institutional molecular screening program (NCT01505400) were evaluated. TP53m were assessed on archived FFPE tumor samples using NGS Illumina MiSeq TruSeq Amplicon Cancer Panel (500x depth of coverage; 10-15% variant detection threshold). Functional effect of TP53m was classified as gain of function (GOF), loss of function (LOF) and variants of unknown significance (VUS), as adapted from IARC TP53 database. Patients' medical records were reviewed for clinical data. TP53m functional effect class was correlated with BC subtypes using Fisher's exact test. TP53m were correlated with time from surgery with curative intent to distant relapse (TTR) and overall survival from diagnosis to death (OS) using Log-rank test. Impact of TP53m functional type on TTR and OS was determined by Cox proportional hazard model.

Results: The study enrolled 220 pts from Oct 2012 - Nov 2015. Median age at diagnosis was 46 years (range 21-80). The cohort included 141 ER+/HER2- (64%), 25 HER2+ (11%) and 54 triple negative (TN) (25%) BC pts. Stage at diagnosis was: I (14%), II (33%), III (24%), IV (21%) and not documented in 8%. Median follow-up was 15 months (m) (range 1-41). Somatic TP53 variants were identified in 80 patients [36%; 23 ER+/HER2- (16%), 18 HER2+ (72%), 39 TN (72%)]. By TP53 functional class, there were 19 GOF (24%), 35 LOF (44%) and 26 VUS (32%). Histologic subtypes were not correlated with TP53m function ($p = 0.09$). TTR for 174 pts, who underwent surgery with curative intent at diagnosis, and OS (94 death events, 43%) are reported in Table 1. TTR and OS were shorter in TP53m compared to TP53 wild type (wt) in the overall cohort and the ER+/HER2- subgroup, but not the HER2+ and TN subgroups.

TABLE 1: Log rank test results		TP53m	TP53wt	P value
Total cohort	TTR (m)	20	54	<0.001
	OS (m)	58	195	<0.001
ER+/HER2-	TTR (m)	29	58	<0.001
	OS (m)	52	208	<0.001
HER2+	TTR (m)	32	94	0.1
	OS (m)	113	275	0.42
TN	TTR (m)	17	18	0.54
	OS (m)	46	49	0.89

In the ER+ subgroup TP53m was significant prognostic factor associated with poor outcome in univariate analysis and remained significant after adjusting for age, stage and grade at diagnosis, neoadjuvant chemotherapy and germline *BRCA1/2* status, with HR for TTR 3.4 (95%CI: 1.9-6.0, $p < 0.001$) and OS 10.2 (95% CI: 4.5-23.4, $p < 0.001$). Within the TP53m group, the functional class of TP53m (GOF, LOF, VUS) was not associated with TTR (23 m, 28 m, 17m, $p = 0.94$) or OS (75 m, 58 m, 49 m, $p = 0.47$).

Conclusions: In advanced breast cancer, somatic TP53m status is prognostic for outcome in ER+/HER2- but not TNBC or HER2+ subtypes. TP53m functional class was not associated with any difference in survival outcomes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-20

Title: Tumor infiltrating lymphocytes in recurrent HER2+ and triple negative breast cancer: Prognostic value according to tumor phenotype

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Body: Background: Tumor infiltrating lymphocytes (TILs) have emerged as a prognostic and potential predictive factor in early triple negative (TN) and HER2+ breast cancer (BC). The prognostic role of TILs in advanced disease is largely unknown.

Methods: 109 HER2+ and TNBC patients with available tumor tissue from regional/distant BC recurrence (collected between 2001 and 2015) were identified from a prospectively maintained database at the Istituto Oncologico Veneto of Padova (Italy). Ipsilateral in-breast relapse/second primaries and contralateral BC were excluded from the definition of recurrence. StrTILs were assessed according to consensus guidelines (Salgado, 2014) on hematoxylin and eosin stained slides from BC recurrence samples and, when available, matched primaries. Post-progression survival was calculated as the time from first BC recurrence to last follow up or death.

Results: StrTILs were evaluable on recurrent BC for 72 cases (HER2+ n=43, TN n=29), after exclusion of lymphnode metastases. Median time to recurrence from initial BC diagnosis was longer for HER2+ than TN cases: 37 months (95%CI 23-51) and 18 months (95%CI 13-23), respectively. Accordingly, median time to biopsy of recurrence from initial BC diagnosis was 43 months (95%CI 35-51) for HER2+ patients and 20 months (95%CI 9-31) for TN patients. Site of biopsy was visceral metastasis in 54% and soft tissue metastasis in 46% of cases (similar for HER2+ and TN).

Median StrTILs level on recurrence was 5% (Q1 2,5%, Q3 10%), without differences according to TN or HER2+ phenotype (Student's t-test p=0.5).

In the whole cohort, post-progression survival did not differ for patients with high (>10%) vs low (\leq 10%) StrTILs on recurrence (HR 0.83 95% CI 0.38-1.80, p=0.64).

In the TN subgroup, high StrTILs on recurrence were associated to a better post-progression survival (median not reached vs 12.7 months for StrTILs >10% and \leq 10%, respectively, HR 0.03 95%CI 0.00-3.64, log-rank p=0.019). To the opposite, in the HER2+ subgroup, high StrTILs were associated to worse post-progression survival compared to low StrTILs (median 27.7 vs 41.1 months for high vs low StrTILs, HR 2.93 95%CI 1.17-7.31, log-rank p=0.016). Test for interaction between tumor phenotype and StrTILs was p=0.15. Similar results were obtained when including only those patients maintaining a concordant TN or HER2+ phenotype on both primary tumor and recurrence (n=59).

StrTILs were assessed on matched primary tumors for 43 patients. Overall, no significant StrTILs variation between primary tumor and recurrence was observed (mean change -4.5%, Wilcoxon p=0.5). Mean change was -2.5% and -7% in HER2+ and TN cases (Wilcoxon p=0.63 and p=0.15, respectively). For TN patients with StrTILs \leq 10% on recurrence, a significant reduction from the primary tumor was observed (mean StrTILs 16% and 4% on primary and recurrent BC, respectively, Wilcoxon p 0.008).

Conclusions: Levels of StrTILs on recurrent BC seem to have an opposite effect on prognosis of metastatic BC patients according to tumor phenotype. Immunohistochemical characterization of TILs is ongoing, data will be available for the meeting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-21

Title: Predictive value of quantitative HER2 and HER3 levels combined with downstream signaling markers in HER2-positive advanced breast cancer patients treated with lapatinib

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Body: Background: Clinical correlates of lapatinib resistance have not been well defined. Previous studies implicated genes regulated by the estrogen receptor (ER) and activation or mutation of proteins downstream from HER family receptors. In the current study, HER2 and HER3 expression levels were quantitatively measured using a VeraTag® fluorescence-based assay, in addition to seven downstream signaling proteins determined by IHC. All biomarkers were correlated with overall survival (OS) in patients treated with lapatinib.

Methods: Formalin-fixed, paraffin-embedded samples were obtained from the primary tumor of 191 patients treated with lapatinib plus capecitabine following progression on trastuzumab. The HERmark® Breast Cancer Assay (Monogram Biosciences, South San Francisco) was used to quantify HER2 protein expression levels. HER3 protein expression was quantified using the VeraTag® technology (Monogram Biosciences). Expression of ER, PTEN, Cyclin E, HIF-2alpha, p-p70S6K, p-AMPK and p-MAPK were determined by IHC (Duchnowska et al., *Oncotarget* 2016; 7:550). OS analyses of HER2 and HER3 were stratified by key clinical variables, including stage and presence of a brain metastasis prior to lapatinib-based therapy.

Results: Among the downstream signaling molecules, HIF-2alpha ($r = -0.23$; $p = 0.047$) and ER ($r = -0.27$; $p = 0.005$) were negatively correlated with HER2 expression after adjustment for multiple testing. PTEN appeared to correlate with HER3, but was not significant after adjustment for multiple testing. OS was significantly shorter for both those below the cut-off level of positivity by the HERmark assay (HR = 1.8; $p = 0.029$), and those with above median HER2 levels (HR = 1.7; $p = 0.009$), as compared to cases with in between levels. The relationship between HER2 and OS is also captured by a U-shaped, parabolic function in HER2 ($p = 0.005$). Elevated HER3 showed a trend toward a correlation with longer OS (HR = 0.66/log; $p = 0.16$), somewhat stronger in the ER-negative subset (HR = 0.55/log; $p = 0.085$) and in the subset with above-median HER2 (0.48/log; $p = 0.10$), where inhibiting HER2 activation of HER3 may be more important. In multivariate Cox models, HER2 (parabola, intermediate HER2 best, $p = 0.001$), presence of brain metastases (HR = 2; $p < 0.001$), ER (HR = 0.60; $p = 0.009$) and either p-p70S6K (HR = 0.66; $p = 0.018$) or p-AMPK (HR = 0.67; $p = 0.022$) were significantly associated with OS (p-p70S6K and p-AMPK were mutually correlated).

Conclusions: Patients with moderately increased HER2 levels may have best outcomes while receiving lapatinib following progression on trastuzumab. This supports recent findings of a less benefit from lapatinib in patients with high HER2 expression (Nunciforo et al., SABCs 2015, **P3-07-08**). HER3 levels do not seem to substantially impact the prognosis. Further studies are warranted to explore the predictive utility of quantitative HER2 and HER3 in guiding HER2-directed therapies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-22

Title: Prognostic significance of nuclear factor kappa B in node-negative breast cancer is most pronounced in luminal B breast cancer

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Body: Background: The importance of the cellular as well as the humoral immune system is increasingly recognized in breast cancer. The transcription factor nuclear factor kappa B (NFKB1) plays an important role in immune cell development. We examined the prognostic significance of NFKB1 in node-negative breast cancer.

Methods: Microarray based gene-expression data for NFKB1 (209239_at) were analysed in four previously published cohorts (Mainz, Rotterdam, Transbig, Yu) of node-negative breast cancer patients not treated with adjuvant therapy (n=824). A meta-analysis of previously published cohorts was performed using a random effects model. Intrinsic subtypes were determined using gene-expression data. Prognostic significance of NFKB1 for metastasis-free survival (MFS) was examined in the whole cohort, in estrogen receptor (ER) positive as well as ER negative patients, and in different intrinsic subtypes: luminal A, luminal B, basal-like, HER2-enriched (HER2-E), and normal-like. Independent prognostic relevance was analysed using multivariate Cox regression.

Results: Higher RNA expression of NFKB1 was related to better MFS in a meta-analysis of the whole cohort (HR 0.48, 95% CI 0.34-0.67, $P < 0.0001$). NFKB1 was significantly associated with MFS only in ER positive (HR 0.41, 95% CI 0.24–0.69, $P = 0.0008$) but not in ER negative (HR 0.76, 95% CI 0.39–1.48, $P = 0.4245$) patients. Regarding intrinsic subtypes, the prognostic significance of NFKB1 was most pronounced in luminal B breast cancer (HR 0.40, 95% CI 0.17–0.93, $P = 0.0338$) as compared to luminal A (HR 0.67, 95% CI 0.16–2.73, $P = 0.5738$), basal-like (HR 0.67, 95% CI 0.29–1.56, $P = 0.3484$), HER2-E (HR 0.70, 95% CI 0.25–2.00, $P = 0.5083$), and normal-like (HR 0.49, 95% CI 0.18–1.32, $P = 0.1579$) breast cancer. However, NFKB1 failed to show independent prognostic significance (HR 0.72, 95% CI 0.42-1.24, $P = 0.235$) in multivariate analysis. Only histological grade of differentiation (HR 2.06, 95% CI 1.27-3.35, $P = 0.003$) and tumor size (HR 1.58, 95% CI 1.01-2.48, $P = 0.045$), but neither age nor HER2 status nor hormone receptor status maintained an independent prognostic association with MFS.

Conclusions: The transcription factor NFKB1 shows prognostic significance in node-negative breast cancer. Higher expression of NFKB1 is associated with improved outcome. The prognostic impact of NFKB1 differs between intrinsic subtypes and is most pronounced in luminal B breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-23

Title: EP3-receptor expression is a prognostic marker for overall survival and progression-free survival in sporadic breast cancer

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Body: Aim: Prostaglandins are tissue hormones with a variety of biological effects and are mainly associated with infection and inflammation. However, elevated prostaglandin synthesis, especially of prostaglandin E₂ (PGE₂) caused by overexpression of cyclooxygenase-2 (COX-2), has also been associated with the development and progression of different kinds of cancer. Clinical trials have shown the potential of non-steroidal anti-inflammatory drugs or specific COX-2 inhibitors (coxibs) in prevention and treatment of malignant disease, as they reduce prostaglandin levels via inhibition of COX-2. Unfortunately, the clinical use of these drugs is limited due to their various side effects. PGE₂ exerts its effects by signaling through four specific membrane-bound receptors, the EP-receptors 1-4. In recent research, the relevance of EP-receptors in carcinogenesis is investigated in the attempt to find a more specific target for the reduction of prostaglandin-effects without inducing side effects. This study evaluates the expression of EP3-receptor on breast cancer tissue and its correlation to progression and survival

Material and methods: A total of 277 sporadic breast cancer samples without primary distant metastases were immunohistochemically analyzed for EP3-receptor expression. Tissue samples were stained with primary anti-EP3 antibodies (monoclonal rabbit IgG). EP3-receptor-expression was quantified by the semi-quantitative immunoreactivity score (IRS); samples with an IRS ≥ 2 were scored as EP3 positive. Statistical analyses were performed with SPSS software using chi-squared test as well as Kaplan-Meier-estimates and Cox regression for survival analyses.

Results: EP3-receptor was expressed in 71.1 % of all cases. EP3-receptor expression did not correlate with clinicopathological parameters such as tumor size or lymph node status at primary diagnosis or with the expression of other immunohistochemical markers (estrogen and progesterone receptor, Her2). Distant metastasis occurred in 49.1 % of EP3 negative cases but only in 32.8 % of EP3 positive cases in an observation period of up to ten years ($p = 0.03$). EP3 receptor positive cases also showed significantly improved progression-free survival rates (overall [$p = 0.01$], ten years [$p = 0.002$] and five years [$p = 0.04$]). Furthermore, EP3-receptor positivity was associated with an improved overall survival rate ($p = 0.002$) and ten years survival rate ($p = 0.001$), whereas short-time survival rate (five years) did not differ between both EP3-groups ($p = 0.10$). In a multivariate analysis comparing all factors with significant influence in univariate testing, EP3-receptor could be confirmed as an independent factor.

Discussion: Our results show that EP3-receptor positivity is a relevant prognostic factor in sporadic breast cancer. Its correlation with a favorable course of disease is especially interesting as EP3 is known to regulate uPA, a well-known parameter which is – on the contrary - associated with unfavorable survival in breast cancer. Therefore, ongoing studies by our group aim to examine the correlation of EP3-receptor to the uPA/PAI1-pathway and to evaluate the possibility to target EP3-receptor in future anti-tumor therapy in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-24

Title: Prognostic value of circulating *PIK3CA* mutations revealed with digital PCR in patients with HER2-positive advanced breast cancer: Results of West Japan Oncology Group study 6110BTR

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Body: Background: Evolution and heterogeneity are the hallmarks of cancer that confer a survival advantage under various stresses, including chemotherapy. However, determining the molecular profile of individual tumors requires biopsies of each site upon recurrence, which is often impractical. Use of a liquid biopsy of circulating DNA in the peripheral blood of cancer patients shows potential for obtaining gene profiles rapidly. In this study, we evaluated the gene status of the primary tumor site and circulating cell-free DNA after recurrence from the serum of patients with HER2-positive breast cancer. This cancer is generally resistant to HER2-targeted therapy due to *PIK3CA* mutations and loss of HER2 amplification.

Methods: Fifty patients with HER2-positive advanced breast cancer previously treated with trastuzumab and taxane were enrolled and treated with either trastuzumab or lapatinib in combination with capecitabine in West Japan Oncology Group (WJOG) study 6110BTR. *PIK3CA* mutation status and HER2 copy number was assessed and compared among 41 tissue specimens of the primary tumor and 35 serum samples after recurrence using digital PCR.

Results: HER2 copy number gain and a *PIK3CA* mutation were present in 61% (25/38) and 33% (13/39) of the primary tumor tissue samples, and in 23% (8/35) and 23% (8/35) of the serum samples after recurrence, respectively. Among the 26 matched tumor and serum samples, concordance of HER2 copy number and *PIK3CA* mutation status was 50% and 85%, respectively. Median follow-up was 44 months. Median OS and PFS was 15.5 (95%CI: 4.67 – undefined) and 5.1 months (95%CI: 2.53-8.35) for pts with the *PIK3CA* mutations and undefined and 6.5 months (95%CI: 4.41 – 9.90) for non-mutated pts, respectively. The mutated pts had shorter overall survival (p value = 0.0709) and progression free survival (HR=0.43 [0.16 – 1.16], p value = 0.0962).

Conclusions: The high concordance rate of *PIK3CA* gene mutation status between primary tumor and serum samples after recurrence was observed and the genetic analysis of circulating cell-free DNA using digital PCR was demonstrated to be a feasible and reliable method for predicting prognosis in the current population. A trend for worse OS and PFS was observed in mutated pts treated with HER2-targeted therapy in combination with capecitabine.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-25

Title: Predictive value of ultra-high *ESR1* mRNA expression in early breast cancer

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Body: Background

Quantitative determination of estrogen receptor mRNA expression in luminal breast tumors is predictive for benefit from adjuvant tamoxifen compared to placebo treatment as has been shown in the large randomized NSABP B-14 trial, while protein determination by IHC or LBA is not (Kim et al JCO 2011). Interestingly, the ultrahigh expression of *ESR1* mRNA (above ER score 10 by Oncotype test) has been indicative for tamoxifen benefit. This predictive cut-off value of mRNA expression is significantly higher than the diagnostic cut-off (at ER score 6.5). Here we tested wether the ultrahigh expression of *ESR1* mRNA determined by commercial MammaTyper® testing is predictive for survival after neoadjuvant chemotherapy treatment of advanced breast cancer.

Materials and Methods

Pretreatment core cut biopsies from n=54 patients with PBC treated within a randomized phase II trial (2) of anthracycline/taxane based NAC with available clinical follow-up information were examined. RNA was extracted from the FFPE sections and *ESR1* mRNA from each section was measured by commercial assays. For technical comparison of *ESR1* mRNA values by Oncotype DX versus MammaTyper® from n=113 surgical samples were analyzed by both commercial assays in a blinded fashion. Statistical analysis was performed using the SAS JMP® 9.0.0 software.

Results

Quantification of *ESR1* mRNA expression after RNA extraction from separate slices of 113 primary breast tumors and determination by different commercial RT-qPCR assays resulted in high correlation of continuous expression results (Spearman $r=0,85$; $p<0,0001$). The rate of *ESR1* mRNA negative cases by both methods by predefined diagnostic cut-offs was low in this cohort (1/113 and 6/113, respectively) resulting in high concordance for positive ER status by both methods. The median expression of ER score and *ESR1* 40-DDCq was high (10,2 and 39,8, respectively) and almost exactly at the predictive ER score cut-off. Hence, the Tamoxifen benefit cut-off of ER score 10 by Oncotype is comparable with a 40-DDCq value of 39,6 for *ESR1* mRNA determination by MammaTyper®, which resembles an *ESR1* mRNA expression 3fold above the diagnostic cut-off. In the independent chemotherapy cohort the optimal discrimination for overall survival could be achieved by an elevated *ESR1* mRNA expression exactly at 39,6 resulting in 100% overall survival for ultra-high expressors and 75 % overall survival for lower *ESR1* mRNA expression after 5 years ($p=0,006$).

Conclusion

Previous data suggest that ultrahigh expression of *ESR1* mRNA is predictive for improved overall survival and tamoxifen benefit (1). Here we show that ultrahigh expression of *ESR1* mRNA is also prognostic in more advanced breast tumors after neoadjuvant chemotherapy. These findings validate the importance of quantitative determination of estrogen receptor expression and substantiate the understanding of receptor expression being a continuous determinant with indication specific cut-off values. Ultrahigh expression of *ESR1* seems to identify a distinct subset of luminal breast tumors with superior prognosis and benefit from tamoxifen treatment. These findings warrant further investigation, which are currently being done in independent large breast cancer cohorts.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-26

Title: uPA/PAI-1 and adjuvant chemotherapy decision-making in early breast cancer

Deluche E, Venat-Bouvet L, Leobon S, Fermeaux V, Aubard Y, Saïdi N, Jammet I and Tubiana-Mathieu N. University Hospital, Limoges, France and Mother and Child Hospital, Limoges, France.

Body: Background: Adjuvant chemotherapy decisions are relatively simple using clinicopathological parameters (Goldhirsch et al. 2007), some cases require the addition of biomarkers as in patients with node negative or micrometastatic (N0), grade II (GII) breast cancer. The objective of this study was to evaluate the impact of uPA/PAI-1 compared to classical criteria +/- Ki67 in adjuvant treatment decision-making in this population.

Methods: This retrospective study included patients, treated from March 2008 to May 2016 in the Department of Medical Oncology at Limoges University Hospital (France). Ki67 and uPA/PAI-1 were analyzed on the initial specimen tumor. Optimal cut-off of Ki67 was defined at 20% (Coates et al. 2015). The positivity thresholds of uPA and PAI-1 were 3 ng/mg and 14 ng/mg respectively (Look et al. 2002). A positive uPA/PAI-1 level was defined as the elevation of at least one of these markers above the positivity threshold. All clinicopathological parameters were recorded prospectively.

Results: 2364 patients with breast cancer were screened. Among these patients, 256 N0, GII breast cancer patients were included with a median age of 62 years (32-87). Discordance between the two markers uPA/PAI-1 and Ki67 was observed in 58% of tumors. Adding Ki67 to the other classical clinicopathological parameters, 143 cases were defined as high risk versus 111 cases without Ki67; low risk were defined in 113 cases versus 145 cases without Ki67. Considering Ki67 status, the indication to perform adjuvant chemotherapy was increased by 29% compared to clinicopathological parameters. uPA/PAI-1 level was positive in these 143 high risk tumors in 93 cases and negative in 50 cases; in the 113 low risk tumors uPA/PAI-1 was positive in 72 cases and negative in 41 cases. uPA/PAI-1 increased indication to perform adjuvant chemotherapy by 15% compared to St Gallen criteria (including Ki67) and by 49% compared to St Gallen criteria (excluding Ki67). Using these two markers the final decision of chemotherapy by the multidisciplinary board was increased by 3% compared to St Gallen criteria (including Ki67).

Conclusions: This study highlighted that uPA/PAI-1 and Ki67 assessment provides additional information for adjuvant chemotherapy decision-making. This study confirms the difficulty to assess the level of importance (weighting) of these biomarkers in adjuvant chemotherapy decision-making. The use of uPA/PAI-1 was feasible, cost-effective in N0, GII breast cancer's patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-27

Title: Baseline serum CA15-3 levels are associated with prognosis for breast cancer patients with non-complete pathological response to neoadjuvant chemotherapy

Fujimoto Y, Imamura M, Higuchi T, Nishimukai A, Yanai A, Miyagawa Y, Murase K, Takatsuka Y and Miyoshi Y. Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.

Body: Background: It has been well demonstrated that patients who achieved pathological complete response (pCR) after neoadjuvant chemotherapy (NAC) had a favorable prognosis compared with patients who did not (non-pCR). Even though pCR was not attained, reduction in tumor volume after chemotherapy may be associated with improved prognosis for a certain number of patients. However, the association between residual tumor volume and prognosis is not necessarily consistent. In order to identify substitute markers for breast cancer patients with non-pCR after NAC, we investigated the impact of serum levels of carcinoembryonic antigen (CEA) and carbohydrate antigen (CA15-3) at baseline as well as post-NAC.

Patients and Methods: Ninety-six breast cancer patients treated with NAC and operated on at the Hyogo College of Medicine were recruited for this study. Serum CEA and CA15-3 were measured prior to chemotherapy as well as at completion of pre-operative treatment. The optimal cutoff points for CEA (1.55ng/ml, normal range: <5.0ng/ml) and CA15-3 (13.25U/ml, normal range: <28.0U/ml) for relapse-free survival (RFS) were determined by analyzing the area under receiver operating characteristic curves in another study involving 613 breast cancer patients. Expression levels of Ki67 in samples obtained at pre- and post-NAC were also determined by means of immunohistochemical staining. Pathological complete response was classified as the absence of residual invasive cancer in the breast and lymph nodes. During a 2.13 years median follow-up period, 15 patients suffered relapse.

Results: pCR and non-pCR was attained by 21 and 75 patients, respectively. For the non-pCR patients, serum CEA levels at baseline were classified into high (n=35) and low (n=38) and serum CA15-3 levels at baseline into high (n=31) and low (n=43). RFS of non-pCR patients with high serum CA15-3 levels was significantly worse than of those with low levels (3-year RFS: 0.47 vs 0.93; p=0.0009). RFS for patients with high and low serum levels of CA15-3 after NAC was also significantly different (p=0.037). As for CEA, no significant association with RFS was observed either at baseline or post-NAC. Univariate analysis demonstrated that tumor size and baseline CA15-3 were significant prognostic factors for RFS. Multivariate analysis showed that both tumor size (hazard ratio (HR): 3.88, 95% confidence interval (CI): 1.21-12.35, p=0.023) and baseline CA15-3 (HR: 13.51, 95% CI: 1.74-105.08, p=0.013) were significant and independent risk factors for relapse. As for lymph node metastasis, tumor grade, residual tumor size and pre- and post-NAC Ki67 expression levels of patients with non-pCR showed no significant association with RFS.

Conclusion and discussion: High levels of serum CA15-3 at baseline constituted a significantly worse prognosis for breast cancer patients with non-pCR. Tumor size at baseline but not residual size and baseline CA15-3 seems to be suitable as a substitute for prediction of outcome for patients with non-pCR. Our findings suggest that these markers may be useful for identifying patients with poor prognosis who may be candidates for additional adjuvant treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-28

Title: BCL2 is a prognostic marker for subtype specific breast cancer

Kim HS, Eom YH, Song BJ and Chae BJ. Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea.

Body: Background

B-Cell lymphoma/leukemia 2 (BCL2) is an anti-apoptosis protein and an important clinical breast cancer prognostic marker. As the role of BCL2 is dependent on estrogen receptor (ER) status, this effect might differ in molecular subtypes. Patients with BCL2 positive expression in breast cancer have a better prognosis based on overall survival (OS) and relapse free survival (RFS). The frequency of BCL2 expression differs among molecular subtypes of breast cancer and it has a favorable prognostic effect. The aim of this study was to evaluate the relationship between prognostic outcome and BCL2 among the molecular subtypes.

Methods

We retrieved data of 1,356 patients in the breast cancer center of Seoul St. Mary's Hospital between 2006 and 2011. Expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), Ki-67, and BCL2 expression was examined by immunohistochemistry. We classified breast cancer into five molecular subtypes based on the 13th St. Gallen immunohistochemical classification, including luminal A, luminal B (HER2 negative), luminal B (HER2 positive), HER2 overexpression, and triple negative. We analyzed the clinicopathological features and assessed the correlation between BCL2 and clinical outcomes, such as relapse free survival (RFS) and disease specific survival (DSS) according to the five molecular subtypes.

Results

Of the 1,124 patients, 519 (46.2%) were BCL2 negative and 605 (53.8%) were BCL2 positive. BCL2-positive expression was associated with young age (<50 years old) ($p=0.036$), lower histological grade ($p<0.001$), low Ki-67 level (<14%) ($p<0.001$), hormone receptor positive ($p<0.001$), HER2 negative ($p<0.001$), luminal breast cancer ($p<0.001$), and low rate of recurrence ($p=0.016$). BCL2-positive expression was also associated with favorable 5-year RFS and DSS ($p=0.008$, 91.4%; $p=0.036$, 95.6%, respectively) in among all patients. BCL2-positive expression in the luminal A breast cancer showed significantly favorable 5-year RFS and DSS ($p=0.023$; $p=0.041$, respectively). However, BCL2 expression was not associated with the prognosis in the other subtypes. The multivariate analysis revealed that tumor size, lymph node metastasis, and BCL2 expression were independent prognostic factors for RFS (BCL2: HR, 0.53; 95% CI, 0.29–0.97; $p=0.015$) and DSS (BCL2: HR, 0.59; 95% CI, 0.08–0.73; $p=0.021$) in luminal A breast cancer.

Conclusion

BCL2 expressed different frequency and played different prognostic roles according to breast cancer molecular subtype. BCL2 is an independent favorable prognostic marker for only luminal A breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-38

Title: Spectral CT based radiomics signature: A potential biomarker for preoperative prediction of lymph node metastasis in breast cancer

Dong D, Zhang X, Fang M, Shen J and Tian J. Institute of Automation, Chinese Academy of Sciences, Beijing, China and Sun Yet-Sun Memorial Hospital, Sun Yet-Sun University, Guangzhou, China.

Body: Purpose:

To investigate the usefulness of radiomics signature based on computed tomographic (CT) spectral imaging, during the late arterial phase (AP) and portal venous phase (PVP), in preoperative predicting the lymph node (LN) metastasis in breast cancer (BC).

patients and methods:

This retrospective study was institutional review board approved, and written informed consent was obtained from all patients. We examined 60 female patients (LN metastasis positivity was 50%) with CT spectral imaging during the AP and the PVP and data was gathered from 2014 to 2016. Excised lymph nodes were located and labeled during surgery according to location on preoperative CT images and were evaluated histopathologically. For each patient, two 3D Hounsfield Unit (HU) gradient maps which revealed the HU change of each voxel were built by quadratic fitting the spectral HU curves during the two phases respectively. Then the radiomics features were then extracted from the regions of BC and a suspicious LN judged manually in these maps. The potential association of the four groups of radiomics features with LN status was assessed by using a Mann-Whitney U test. The area under curves (AUC) of the receiver operating characteristic curves (ROC) were compared with data obtained from the conventional CT image.

results:

The 3D HU gradient map showed a great power of distinguishing among different components and was considered as a more effective tool for revealing the intratumour heterogeneity than the conventional CT image since the slope of spectral HU curves were significantly higher in malignant tumor. More than 500 radiomics features extracted from the regions of LN during the AP and the PVP exhibited significant differences ($P < 0.05$). Moreover, the numbers of this kind of features extracted from the regions of BC were more than 200. The highest AUC of single feature was 0.70, which was higher than those from the conventional CT image.

Conclusion:

Quantitative radiomics features based on 3D HU gradient maps have the potential to be exploited as an effective biomarker for preoperative prediction of lymph node metastasis in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-29

Title: The molecular subtype has greater influence on prognosis of breast cancer than age at diagnosis

Kim HS, Eom YH, Song BJ and Chae BJ. Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea.

Body: Introduction

Young age breast cancers have been considered more aggressive and associated with a poor prognostic factors. But, it remains controversial about the prognostic value of age at diagnosis in breast cancer. While some studies have found that young patients have worse clinical outcomes, others report young patients have a more favorable prognosis, or that there is no relationship between prognosis and age. The purpose of this study was to assess clinical, pathological variables, and different prognosis across three different age cohorts. and we also aimed to identify whether the distribution of molecular subtypes brings a different prognosis at different age cohorts.

Methods

Our study retrieved data from 1,410 patients with primary breast cancer who underwent surgery and the adjuvant treatment in the Department of Surgery, The Catholic University of Korea College of Medicine, Seoul St. Mary's Hospital between January 2007 and December 2011 were retrospectively analyzed. Our study classifies each molecular subtype in three age cohorts including ages <40, 40-50, >50 years. The association of age and molecular subtypes with recurrence free survival (RFS) and disease specific survival (DSS) were analyzed.

Result

Of 1,138 patients, 152 (13.4%) were younger than 40 years, 461 (40.5%) were between 40 and 50 years, and 525 (46.1%) were older than 50 years. The group of younger patients (age <40 years) had association with poor prognostic factors, such as higher cancer stage, higher rate of lymph node metastasis, lower ER and PR expression, and higher ki-67 ($\geq 14\%$) than older patients (age 40-50 and age >50 group). Patients aged <40 years had higher proportion of triple negative breast cancer ($p < 0.001$), and had significantly shorter RFS than patients aged 40-50 years and >50 years ($p = 0.047$). There was no statistically difference in DSS ($p = 0.412$). In subgroup analysis according to molecular subtypes, inferior RFS was observed for patients aged <40 years with only luminal A breast cancer ($p = 0.017$). No difference according to molecular subtypes was observed in DSS. Aged <40 years is a poor prognostic factor only in luminal A breast cancer, so it has limited prognostic value. It is thought to be due to longer exposure to the high level of estrogen in luminal A breast cancer patients aged <40 years.

Conclusion

Our findings suggest that the molecular subtype is the more main determinant of outcome than age at diagnosis in breast cancer. Young age at diagnosis is not a poor prognostic factor for all breast cancer patients, and it is a restrictive prognostic factor of recurrence in luminal A breast cancer. It is necessary to consider the aggressive and extended hormonal treatment to improve outcomes for young patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-30

Title: OncotypeDX® for breast cancer: A multigene assay that makes a difference?

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Body: Objective: OncotypeDX® (ODX) is a multigene diagnostic assay that can estimate the 10 year-risk of distant recurrence in women with hormone receptor positive (HR+) and node negative (N-) early breast cancer. The test reports a Recurrence Score® (RS) and three risk group categories have been described: low-risk (<18), intermediate-risk (18-30) and high-risk (≥31). It helps the oncologist in the adjuvant chemotherapy decision process and globally leads to a reduction in the recommendation for chemotherapy use. This test is expensive and represents an economic burden in a publicly funded province. Nonetheless, its use has been approved over other gene expression profiling like MammaPrint® based on the evidence of its prognostic and predictive ability. We evaluated the adequacy of the requests for the ODX in an academic setting after the introduction in May 2012 of a reference framework for its use in Québec, Canada and the impact on chemotherapy recommendation. The costs generated by the test were also determined. **Methods:** We included all patients with an ODX request from two University Centers, CICM and CHUS, and estimated the concordance with the current provincial guideline for which an ODX may be ordered (invasive breast cancer HR+/Her2-/N- that is T1b with unfavorable characteristics or T1c or T2). For the intermediate-risk group, the factors influencing the final decision to use systemic chemotherapy were analysed. The projected cost-effectiveness of the ODX was derived from the proportion of patients (pts) for which the chemotherapy was not recommended. **Results:** Between May 2012 and December 2014, a total of 201 pts, 123 pts from CICM and 78 from CHUS, had an ODX done. In 93,0% (95%CI, 89,5-96,6) of pts, ODX was ordered correctly with respect to the guideline. There was no statistical differences between both sites (CICM: 92,7% [95%CI, 97,3-88,1]; CHUS 93,6% [95%CI, 88,2-99,0]). A total of 9 pts had high-risk RS (4,5%), 78 pts had intermediate-risk RS (38,8%) and 112 pts had low-risk RS (55,7%). Chemotherapy was recommended for 31 pts (18,2%) instead of an estimated 58,0% prior to the use of ODX according to previous reports published. In the intermediate-risk group, the majority of pts (74,4%) did not receive chemotherapy. The patient's preference and the absence of a proven benefit were the main reasons for withholding chemotherapy in this group. The additional cost associated with the use of the ODX was compensated with the reduction of the adjuvant systemic chemotherapy prescribed and its derived expenses (chemotherapy cost, nursing time and hospitalisations) and savings of 100 K were observed. **Conclusions:** In early breast cancer HR+ and N-, the use of ODX in two University Hospitals is concordant with published recommendations. ODX use is cost effective. This benefit does not take into account the psychological burden that comes with the decision to use adjuvant chemotherapy; neither does it evaluate potential long term complications. The widespread use of ODX must be looked at critically in face of other emerging gene signature tests like Endopredict® and PAM50®. As for the predictive ability of the ODX for adjuvant chemotherapy, one can question the strength of the actual evidence and argue if it confers this test an advantage over other multigene assays.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-31

Title: High-risk breast cancer is less likely to occur in the ductal hyperplasia lesions or low-grade DCIS ~ Consideration of long-term follow-up observation after surgical biopsy

Takebe K and Arai T. Takebe Breast Care Clinic, Takamatsu, Kagawa, Japan.

Body: <Introduction>

Even if many ductal hyperplasia lesions and low grade DCIS are detected by breast cancer screening using high-resolution imaging modalities, we have often experienced high-grade breast cancer as an interval cancer. We might consider whether the detection of ductal hyperplasia lesions and low grade DCIS in breast cancer screening really leads to a decrease in breast cancer death. Therefore, we examined the follow-up periods after the removal of lesions. And we would like to refer to the problem of over-diagnosis and over-treatment of LGDCIS.

<Subjects and Methods>

At our facility from 1997 to 2012, we examined 354 cases of ductal hyperplasia lesions which were treated with surgical biopsy and followed up with observations spanning at least three years. The ductal hyperplasia lesions shown here are the usual ductal hyperplasia, flat epithelial atypia, atypical ductal hyperplasia, and intraductal papilloma. The median of the follow-up observation period was 73 months. In addition, we had 153 cases of low-grade DCIS patients who underwent follow-up of more than three years without postoperative irradiation. The median of the follow-up period was 63 months.

<Result>

(1) In ductal hyperplasia lesions, 25 cancers occurred in both breasts. There were 13 cases of DCIS in the histopathological examination (52%) and 12 cases of invasive cancer (46%). In DCIS, low-grade DCIS of Van Nuys1 accounted for 86%. Luminal A was 75% in invasive cancer. Lymph node metastasis was observed in only two cases, with one case having one metastatic lymph node and the other having two. Her2 type and triple-negative type accounted for one case each. Her2 type was microinvasive carcinoma, and Ki67 was 22% in triple negative type. The potential for malignancy in both was not so high.

In low-grade DCIS (non-irradiated), 11 cancers occurred in both breasts. There were 8 cases of DCIS (73%) and three cases of invasive cancer (27%). All of the DCIS cases were VanNuys1. All of the invasive cancer cases were luminal A.

(2) We compared the cancer occurrence in benign lesions and low-grade DCIS (surgery side). The occurrence rate was 2.2% for benign lesions and 3.2% for low-grade DCIS over a five-year period. There was no significant difference found between the two groups.

<Conclusion> High-risk breast cancer is less likely to occur in ductal hyperplasia lesions or low-grade DCIS. In other words, terminal duct lobular unit (TDLU) in both breast who had been affected with ductal hyperplasia or low grade DCIS lost a risk of the occurrence of high grade malignant cancer. Therefore, women with ductal hyperplasia and LGDCIS should be placed in the low-risk group of future breast cancer deaths. Small low grade DCIS might be regarded to be nearly as innocent as benign hyperplastic lesions.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-32

Title: Tissue kallikrein-related peptidase 4, a novel biomarker in triple-negative breast cancer

Dorn J, Yang F, Aubele M, Kiechle M and Schmitt M. Klinikum rechts der Isar, Munich, Germany and Institute of Pathology, Helmholtz Center Munich, Neuherberg, Germany.

Body: Triple-negative breast cancer (TNBC), lacking the steroid hormone receptors estrogen receptor and progesterone receptor and not overexpressing the oncoprotein HER2, is characterized by its aggressive pattern and insensitivity to endocrine and HER2-directed therapy. Human kallikrein-related peptidases KLK1-15 provide a rich source of serine protease-type biomarkers associated with tumor growth and cancer progression for a variety of malignant diseases. In this study, recombinant KLK4 protein was generated and affinity-purified KLK4-directed polyclonal antibody pAb587 established to allow localization of KLK4 protein expression in tumor cell lines and archived formalin-fixed, paraffin-embedded triple-negative breast cancer (TNBC) tumor tissue specimens. For this, KLK4 protein expression was assessed by immunohistochemistry in primary tumor tissue sections (tissue microarrays) of 188 TNBC patients, mainly treated with anthracycline- or CMF-based polychemotherapy. KLK4 protein is localized in the cytoplasm of tumor cells and that of accessory stroma cells. In this patient cohort, elevated stroma KLK4 expression, but not tumor cell expression, is predictive for poor disease-free survival by univariate analysis (hazard ratio: 2.26, $p=0.001$) and multivariable analysis (hazard ratio: 2.12, $p<0.01$). Likewise, univariate analysis revealed a trend for statistical significance of elevated KLK4 stroma cell expression for overall survival of TNBC patients as well.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-33

Title: Refining the prognostic value of Ki67 biomarker by ataxia telangiectasia mutated (ATM) status in a retrospective study of early stage hormone receptor positive breast cancer

Feng X, Li H, Kornarga E, Dean M, Lees-Miller S, Riabowol K, Magliocco A, Morris D, Watson P, Enwere E, Bebb G and Paterson A. Vancouver Island Cancer Center-British Columbia Cancer Agency, Victoria, BC, Canada; Faculty of Medicine, University of British Columbia, Victoria, BC, Canada; Tom Baker Cancer Center, University of Calgary, Calgary, AB, Canada; University of Calgary, Calgary, AB, Canada; Functional Tissue Imaging Unit, Translational Research Laboratory, Tom Baker Cancer Centre, Calgary, AB, Canada; Health Science Building, University of Calgary, Calgary, AB, Canada and H. Lee Moffitt Cancer Center, Tampa, FL.

Body: Purpose: The current study was designed to investigate the combined influence of ataxia telangiectasia mutated (ATM) and Ki67 on clinical outcome in early stage hormone receptor positive breast cancer (ES-HPBC), particularly in patients with smaller tumors (< 4 cm) and fewer than four positive lymph nodes.

Methods: Formalin-fixed paraffin-embedded specimens of resected primary breast tumors from 532 patients diagnosed with early stage breast cancer were used to construct a tissue microarray. Samples from 297 patients were suitable for final statistical analysis. We detected ATM and Ki67 proteins using immunohistochemistry and quantified their expression with digital image analysis. Data on expression levels were subsequently correlated with clinical outcome.

Results: Remarkably, ATM expression was useful to stratify the low Ki67 group into subgroups with better or poorer prognosis. Specifically, in the low Ki67 subgroup defined as having smaller tumors and no positive nodes, patients with high ATM expression showed better outcome than those with low ATM, with estimated survival rates of 96% and 89% respectively at 15 years follow up ($p = 0.04$). Similarly, low-Ki67 patients with smaller tumors, 1-3 positive nodes and high ATM also had significantly better outcomes than their low ATM counterparts, with estimated survival rates of 88% and 46% respectively ($p=0.03$) at 15 years follow up. Multivariable analysis indicated that the combination of high ATM and low Ki67 is prognostic of improved survival, independent of tumor size, grade, and lymph node status ($p = 0.02$).

Low Ki67/high ATM expression independently predicts favorable disease survival in a multivariate model in low Ki67 subgroup of ES-HPBC (Stage I-III)

Variables	p value	HR (95%CI)
Low Ki67/high ATM vs Low Ki67/low ATM	0.02	0.36 (0.15-0.88)
Tumor size (T1/2 vs T3/4)	0.01	0.16 (0.04-0.69)
LN status (- vs +)	0.14	0.49 (0.20-1.25)
LVI (- vs +)	0.16	0.51 (0.20-1.31)
Grade (1/2 vs 3)	<0.0001	0.28 (0.11-0.67)
Age (<65 vs >65)	0.01	0.26 (0.10-0.74)

Conclusions: These data suggest that the prognostic value of Ki67 can be improved by analyzing ATM expression in ES-HPBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-34

Title: Risk stratification with EndoPredict signature for luminal subtype breast cancers: Re-analyzing microarray experiments with Han Chinese origin

Huang C-C, Tsai M-L, Tu S-H and Huang C-S. Cathay General Hospital, Taipei City, Taiwan; Fu-Jen Catholic University, New Taipei City, Taiwan and Taipei Medical University, Taipei City, Taiwan.

Body: Introduction: Breast cancer is a heterogeneous disease in terms of molecular aberrations. Luminal breast cancers, most of which are estrogen receptor (ER) positive without human epidermal growth factor receptor 2 (HER2) over expression clinically, constitute the majority of human breast cancers with better prognosis compared with basal-like or HER2-enriched subtype. The aim of the study is to evaluate the prognostication of EndoPredict signature, for which high- and low-risk group is defined based on a multi-gene assay. EndoPredict signature is supposed to guide adjuvant therapy for ER+/HER2- luminal breast cancers with up to three positive lymph nodes, while the test has not been validated for Han Chinese population yet.

Materials and methods: Our microarray experiments (partially published under GSE48391) and two publicly available microarray studies (GSE5460 and GSE20685) constituted the combined dataset of 565 breast cancers with Han Chinese origin, of which 280 were ER+/HER2- by immunohistochemical analysis. Transformation of Affymetrix microarray gene expression values to RT-qPCR-based expression values were conducted with the mathematical formula provided by the EndoPredict investigators, with gene-specific transformation factor and offset. Each enrolled patient was categorized into either high- or low-risk group based on the result of EndoPredict (EP) algorithm.

Results: Direct adaptation of the EP algorithm for microarray gene expression data resulted in over inflation of EP scores, and most cases were categorized into the high-risk group with the predefined threshold of EP score of 5 and adjustments with rescaling and relocations of microarray-based EP scores were performed. The proportion was 88% for low-risk group and 74% for high-risk group during the 10-year follow up period, with disease-specific survival advantage reported for those with EP-predicted low-risk group patients.

Risk stratification of Taiwanese breast cancers by EP algorithm: disease-specific survival

Risk group	Total number	Failed number	Censored number	Percentage of disease-specific survival censored patients
Low-risk	64	8	56	87.5%
High-risk	156	40	116	74.4%

P=0.01, log-rank test

On the other hand, borderline overall survival discrepancy was observed (89% for low-risk and 80% for high-risk group).

Risk stratification of Taiwanese breast cancers by EP algorithm: overall survival

Risk group	Total number	Failed number	Censored number	Percentage of overall survival censored patients
Low-risk	64	7	57	89%
High-risk	156	31	125	80.1%

P=0.05, log-rank test

In addition, patients with high-risk EP scores were associated with larger tumor size, higher nuclear grade, and more involved lymph nodes.

Discussion and conclusion: The study provides a solution to enhance the comparability between the FFPE/RT-qPCR based EP algorithms and fresh frozen microarray gene expression data. The statistical framework presented here provides an "in-silicon" validation for EP algorithm and further studies taking clinical parameters into consideration will augment the clinical applicability of EP scores and EPclin scores to ascertain the prognostic power of multi-gene assay for luminal breast cancers in Taiwan.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-35

Title: Rs1008805 polymorphism in *CYP19A1* gene is related to the prognosis of early breast cancer

Shao X, Zheng Y, Huang P, Shi L and Wang X. Zhejiang Cancer Hospital, Hangzhou, Zhe jiang, China.

Body: Purpose Aromatase, encoded by *CYP19A1* gene, is a rate-limiting enzyme in the conversion of androgens into estrogens. Given the critical role of *CYP19A1* gene in estrogen synthesis, the potential impact of genetic polymorphisms at *CYP19A1* gene on breast cancer patient survival deserves further study.

Methods Rs1008805 polymorphism in *CYP19A1* gene were genotyped on 406 Chinese Han women with stage I-II and operable stage III breast cancer. Associations were explored between rs1008805 genotypes and disease-free survival (DFS).

Results Totally, there were 200 (49.3%) patients with AA genotype, 169 (41.6%) with AG variant, and 37 (9.1%) carrying GG variant. No significant differences were found in DFS or 5-year survival rate among the whole population with these three genotypes. However, in postmenopausal women, rs1008805 genotypes were significantly associated with DFS and 5-years DFS rate (AA versus AG versus GG: 63.1 months versus 54.3 months versus 13.7 months; 48.6% versus 46.0% versus 14.3%; $P = 0.015$). In addition, women with GG variant had a poorer DFS, 5-years DFS rate when compared with those carrying AG or AA genotype (GG versus AG or AA: 13.7 months versus 56.3 months; 0% versus 52.1%; HR, 2.462; 95 % CI, 1.310-4.628; $P = 0.004$). Being adjusted by patients features in multivariate analyses, GG genotype remained an independent prognostic factor for DFS (HR, 2.706; 95 % CI, 1.393-5.257; $P = 0.003$). Whereas premenopausal women with the homozygous minor allele had a marginally improved DFS, 5-years DFS rate when compared with those carrying the common allele (GG versus AG or AA: 87.0 months versus 48.7 months; 60.3% versus 42.7 %; HR, 0.544; 95% CI, 0.295-1.003; $P = 0.051$). However, there was no relationship between GG genotype and DFS or 5-year DFS when adjusted by patients features in multivariate analyses.

Conclusions The present study indicated that GG genotype of rs1008805 SNP in the first exon of *CYP19A1* gene was significantly related to a worse DFS or 5-years DFS rate in postmenopausal women with early breast cancer. This finding is novel, if confirmed, *CYP19A1* rs1008805 genotypes may turned to be a prognostic biomarker for early breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-36

Title: Clinicopathological predictors for low risk recurrence distinguished by 21-gene recurrence score in estrogen receptor-positive invasive breast cancer patients

Tsuchida Y, Hayashi N, Omata F and Yamauchi H. St. Luke's International Hospital, Tokyo, Chuo-ku, Akashi-cho, Japan and St. Luke's International Hospital Center for Clinical Epidemiology, Tokyo, Chuo-ku, Akashi-cho, Japan.

Body: Background: The 21-gene Recurrence Score (RS) (Oncotype DX®; Genomic Health, Redwood City, CA) is the most valid and reliable multigene assay to predict prognosis or response to treatment in hormone receptor-positive invasive breast cancer patients. However, in Japan, the test is expensive (about 4,000 US dollars) and one of the problems is that about 30% of patients will be categorized as having moderate recurrence risk. If clinicopathologic factors can be used to predict patients with low recurrence, many patients can avoid postoperative chemotherapy without testing the RS. Such predictors shall have a substantial impact on medical economics too. The aim of this study was to determine significant clinicopathological predictors for low recurrence risk by RS in patients with estrogen receptor-positive primary breast cancer.

Methods: Retrospective cross-sectional study was conducted in tertiary referral hospital in Tokyo, Japan. Two hundreds twenty patients with estrogen receptor-positive invasive breast cancer underwent surgery for breast cancer and tested for RS from November 2009 to March 2016. RS ≤ 18 was defined as low recurrence risk. The patients were divided into 2 groups, patients with low recurrence risk (n=143) and with a moderate/high recurrence risk (n=77). Age, menopausal status, histologic type (invasive ductal vs. lobular carcinoma), nuclear grade, progesterone receptor (PR) expression, Ki67 index, tumor size, lymph node status, and lymphovascular invasion were considered as candidate predictors. Student's t test or Wilcoxon-Rank Sum test and Fisher's exact test was used for continuous variables and proportion, respectively. Simple and multiple logistic regression model were used to determine significant predictors. Classification and regression tree analysis (CART) was also conducted.

Results: Mean age (SD) of low and moderate/high recurrence patients was 53 years-old (9.4) and 55 years (10.2), respectively. Univariate analyses revealed that the invasive lobular carcinoma, the high PR expression, Ki67 < 24 , and the absence of lymphovascular invasion were significantly associated with low recurrence risk. According to multiple logistic regression, The odds ratio (OR) [95%CI] of histological type (invasive lobular), high PR expression, Ki67 < 24 , and the absence of lymphovascular invasion was 0.43 [0.08-1.8], 10 [5.4-23.6], 0.95 [0.93-0.97], and 0.57 [0.23-1.18], respectively. The area under the receiver operating characteristic curve was 0.83. CART showed that the probability of low recurrence risk was 79% if with high PR expression, and 92% if with high PR expression and Ki67 < 24 .

Conclusions: High PR expression and Ki67 < 24 were significant predictors for low recurrence risk by RS in estrogen receptor-positive invasive breast cancer patients. More than 90% of patients with high PR expression and Ki67 < 24 could be classified as low recurrence risk by RS.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-05-37

Title: In-situ hybridization of microRNA and support vector machines–based prognostic classifiers for breast cancer

Cao Z, Yao L, Hu X, Zhang Z and Shao Z. Key Laboratory of Breast Cancer in Shanghai, Fudan University Shanghai Cancer Center, Shanghai, China and School of Public Health, Fudan University, Shanghai, China.

Body: Purpose: We plan to use a tissue microarray-based microRNA(miRNA) detection by in situ hybridization with LNA probe and support vector machine on base of data mining to construct the Prognostic model for breast cancer and triple negative breast cancer (TNBC).

Methods: Combining the BreastMark miRNA database and papers published, we chose candidate miRNAs. Then a tissue microarray-based miRNA detection in situ hybridization with LNA probe was used to detect miRNA expression in 445 breast cancer tissue. Univariate analysis with Kaplan-Meier identified independent prognostic factors, 445 patients was divided into the training set and validation set (70% VS 30%) by software R(version 3.2.3), then support vector machine on base of data mining was used to construct the Prognostic model for breast cancer and triple negative breast cancer via software R and predict.svm(e1071).

Results: 15 miRNAs were detected from 445 breast cancer tissue and univariate analysis with Kaplan-Meier identified significant prognostic miRNAs: miR-361-5p, miR-301a, miR-223, miR-421, miR-454, miR-493 were the independent prognostic factors for DFS. By using data mining technique, we combined clinical factors(stage, tumor size, lymphnode status, molecular subtype) and miRNAs mentioned above to establish the personalized predictive mathematical models SVM-BC and SVM-TNBC.

In the training set and validation set of SVM-BC model, the AUC value of ROC curve for DFS were 0.86,0.83. The total accuracy,sensitivity,specificity of SVM-BC model for DFS were 94%,73%,99% and 84%,57%,92% in the training set and validation set. The SVM-BC model can divide breast cancer patients into high-risk and low-risk groups, and 5-year survival rates for low-risk patients were higher than that for high-risk patients(96.8% VS 39.3%, $p<0.001$).

In the training set and validation set of SVM-TNBC model, the AUC value of ROC curve for DFS were 0.84,0.86. The total accuracy,sensitivity,specificity of SVM-TNBC model for DFS were 90%,71%,97% and 84%,55%,94% in the training set and validation set. The SVM-TNBC model can divide breast cancer patients into high-risk and low-risk groups, and 5-year survival rates for low-risk patients were higher than that for high-risk patients(93.9 % VS 23.5%, $p <0.001$).

Conclusion: The Prognostic model basing of tissue microarray-based miRNA detection in situ hybridization with LNA probe and support vector machine for prognosis can effectively predict the prognosis of breast cancer and TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-06-01

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-06-02

Title: De novo and relapsed metastatic breast cancer: Presentation and survival changes over time 1990-2009

Malmgren JA A, Atwood MK K and Kaplan HG G. HealthStat Consulting Inc, Seattle, WA and Swedish Cancer Institute, Seattle, WA.

Body: Background: De novo stage IV and relapsed stage IV metastatic breast cancer (MBC) may present with different biology and respond differentially to treatment. De novo stage IV patients are treatment naive which may confer a survival advantage. Prior radiation and chemotherapy treatment for relapsed stage IV (rMBC) patients reduces available treatment options and may confer a survival disadvantage. The possible survival differences have not been adequately studied.

Methods: A retrospective cohort study of de novo and relapsed stage IV MBC patients from a dedicated institutional BC registry database, years 1990-2009 [invasive BC N = 7653, MBC N=1077 (de novo = 221, relapsed = 856)]. Relapsed stage IV MBC (rMBC) patients were identified by continuous annual follow up of stage I-III patients for distant metastases (M1). De novo stage IV MBC was identified as such at diagnosis using AJCC 7 diagnostic criteria (Any T, Any N, M1). Chi square tests, Kaplan Meier plots, and Cox regression survival analysis were conducted. Disease specific survival (DSS) interval was time from diagnosis date to breast cancer death for de novo MBC and time from first distant relapse date to breast cancer death for rMBC patients.

Results: De novo MBC incidence was constant at 3% per year over time but rMBC decreased from 17% to 8% (1990-99 to 2000-09, $p < .001$). Among rMBC cases, percentage of her2-neu (her2) positive cases decreased 16% over time from 1990-99 to 2000-09. With the decrease in her2 positive cases the relative number of hormone receptor-/her2- (triple negative) rMBC cases increased proportionately. MBC average survival was 3.2 years (.01, 19.67) with median de novo survival equal to 3.81 years and median rMBC survival equal to 1.81 years ($p < .001$). Overall, 5 year DSS among de novo stage IV patients was superior at 43% vs. 21% among rMBC patients ($0 < .001$). Over time de novo stage IV 5 year DSS improved significantly from 31% to 51% ($p = .018$). rMBC 5 year DSS did not improve over time and appeared to decline but not significantly [1990-99 23%, 2000-09 18%, $p = .079$]. In Cox regression analysis, factors associated with worse DSS were TN status (RR = 2.20, 95% CI 1.75, 2.75), age 70+ (RR = 2.35, 95% CI 1.73, 3.19), and rMBC vs. de novo MBC (RR = 2.44, 95% CI 1.95, 3.07).

Conclusions: We observed better overall DSS among de novo MBC patients compared to rMBC patients. DSS rates improved over time in de novo MBC but not among the relapsed MBC patients. Overall, better breast cancer specific survival among de novo MBC patients may be related to better response to treatment in therapy naive de novo MBC and use of neoadjuvant therapy methods. It appears the introduction of targeted her2-neu therapy has reduced the number of Her2+ patients with relapsed MBC. Less effective treatment options for de novo and relapsed triple negative BC and BC in the elderly may be responsible for the elevated risk of mortality from MBC observed among TNBC and elderly patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-06-03

Title: The 3'UTR signature defines a highly metastatic subgroup of triple-negative breast cancer

Wang L, Hu X, Wang P and Shao Z-M. Fudan University Shanghai Cancer Center, Fudan University, Shanghai, China; Shanghai Medical College, Fudan University, Shanghai, China; Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai, China; School of Life Science and Technology, ShanghaiTech University, Shanghai, China and Institutes of Biomedical Sciences, Fudan University, Shanghai, China.

Body: Purpose. Triple-negative breast cancer (TNBC) is a highly heterogeneous disease with an aggressive clinical course. TNBC relapse or metastasis is associated with an unfavorable prognosis so predictive models are needed to chart potential patient outcomes. Alternative polyadenylation (APA) has recently gained attention as a major player influencing the kinetics of gene regulation under diverse physiological and pathological status, resulting in the dynamic usage of 3' untranslated region (3'UTR). We postulate that alternative 3'UTR patterns, if incorporated into a single model, could improve postoperative risk stratification and identify a highly metastatic subgroup of TNBC. In this study, we used alternative 3'UTR patterns to improve postoperative risk stratification.

Experimental Design. We collected 327 publicly available microarrays and randomly stratified samples in a training ($n = 164$) and validation set ($n = 163$). Then, 1,933 3'UTRs were generated based on expression ratios of alternative 3'UTR. After initial feature filtering, we built a 17-3'UTR-based classifier using an elastic net model and validated the prognostic accuracy in both training and validation sets.

Results. Time-dependent ROC comparisons and Kaplan–Meier analyses confirmed an outstanding discriminating power of our prognostic model for TNBC patients. In the training cohort, 5-year event-free survival (EFS) was 78.6% (95% CI 71.2–86.0) for the low-risk group, and 16.3% (95% CI 2.3–30.4) for the high-risk group (log-rank $p < 0.0001$; hazard ratio [HR] 8.29, 95% CI 4.78–14.4). In the validation set, 5-year EFS was 75.6% (95% CI 68.0–83.2) for the low-risk group, and 33.2% (95% CI 17.1–49.3) for the high-risk group (log-rank $p < 0.0001$; HR 3.17, 95% CI 1.66–5.42). The 17-3'UTR-based classifier could be used to independently predict outcomes and this outperformed prognosis using classical clinicopathological risk factors.

Conclusions. Our 17-3'UTR-based classifier provides a superior prognostic performance for estimating disease recurrence and metastasis in TNBC patients and it may permit personalized management strategies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-06-04

Title: Breast cancer after Hodgkin lymphoma: Influence of endogenous and exogenous gonadal hormones on the radiation dose-response relationship

Krul IM M, Opstal - van Winden AWJ WJ, Aleman BMP MP, Janus CPM PM, van Eggermond AM M, de Bruin ML L, Hauptmann M, Krol ADG DG, Schaapveld M, Broeks A, Kooijman KR R, Fase S, Lybeert ML L, Zijlstra JM M, van der Maazen RWM WM, Kesminiene A, Diallo I, de Vathaire F, Russell NS S and van Leeuwen FE E. Netherlands Cancer Institute, Amsterdam, Netherlands; Erasmus University MC Cancer Institute, Rotterdam, Netherlands; Utrecht University, Utrecht, Netherlands; Leiden University Medical Center, Leiden, Netherlands; Catharina Hospital, Eindhoven, Netherlands; VU University Medical Center, Amsterdam, Netherlands; Radboud University Medical Center, Nijmegen, Netherlands; International Agency for Research on Cancer, Lyon, France and Institut Gustave Roussy, Villejuif, France.

Body: Background

After chest radiotherapy (RT) for Hodgkin lymphoma (HL), women experience a dose-dependent increased breast cancer (BC) risk. It is unknown whether endogenous and exogenous gonadal hormones affect the radiation dose-response relationship.

Methods

We conducted a nested case-control study among female 5-year HL survivors treated before 41 years between 1965-2000. Data were collected through medical records and questionnaires for 174 BC cases and 466 matched controls. RT charts, simulation films and mammography reports were used to estimate the radiation dose to the location of the breast tumor.

Results

The median interval between HL and BC diagnosis was 21.9 years. 98% of BC cases had received chest RT, compared to 92% of controls. We observed a linear radiation dose-response curve with an adjusted excess odd ratio (EOR) of 5.4%/Gray (95%CI:1.8%-13.37%). Women with menopause <30 years (caused by high-dose procarbazine or pelvic RT) had a lower BC risk (OR:0.13, 95%CI:0.03-0.54) than women with menopause \geq 50 years. BC risk increased with 7.4% for each additional year of intact ovarian function after RT ($P < 0.001$). Among women with an early menopause (<45 years), the use of hormone replacement therapy (HRT) for \geq 2 years did not increase BC risk (OR:0.81, 95%CI:0.30-2.21). Endogenous and exogenous hormones did not statistically significantly modify the slope of the radiation dose-response relationship.

Conclusion

HRT use did not appear to increase BC risk in female HL survivors with a therapy-induced early menopause. Moreover, there was no evidence for interaction between RT dose and years with intact ovarian function or HRT use.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-06-05

Title: Development and validation of a new non-parametric breast cancer risk assessment model on US and European screening populations

Ragusa S, Gauthier E, Dartois L, Tice J, Dancourt V, Arveux P, Brixi Z, Bernoux A, Soyer P, Delattre H, Brechenade S, Catajar N, Kaufmanis A, Hélin VM, Clavel F, Kerlikowske K, Miglioretti D and Delalogue S. Statlife, Villejuif, France; Centre for Research in Epidemiology and Population Health (CESP), U1018, Team 9, Villejuif, France; University of California San Francisco, San Francisco, CA; ADECA21, Dijon, France; Cote d'Or Registry, Centre Georges François Leclerc, Dijon, France; ADOC94, Charenton Le Pont, France; ADMC91, Briis Sous Forges, France; ADMY78, Versailles, France; ADK92, Puteaux, France; PSVO95, France; CDC93, France; ADC77, France; University of California Davis, Davis, CA, France and Gustave Roussy, Villejuif, France.

Body: Background: Stratified breast cancer (BC) prevention is a major option for the future but requires clinically meaningful internationally validated risk models. Non parametric models may be alternate methods for modeling in very large cohorts. We have previously shown that a non-parametric similarity-based k-nearest neighbors' (kNN) model performs better than the BCRAT/Gail model to on 65 000 women of the E3N French national cohort (Dartois et al 2015). We used this method to develop and validate a mammographic density-based model in larger general screening populations (pops).

Methods: A modified version of a data-mining based algorithm, the kNN method, was implemented and adapted as previously described [ref Dartois]. Core concept of kNN algorithm is to gather similar profiles using a distance computation.

We developed a BC risk prediction model on 629 229 women (wn) from the US Breast Cancer Research Consortium (BCSC), with 5 times random selection of learning and validation sets (75/25 %) within the cohort. 5 parameters were included: age, family history, previous breast biopsy, mammographic density and race. The model's performances (discrimination using c-stat (AUC) and calibration using E/O ratio) were evaluated and compared to the parametric model developed on the same pop (param/BCSC)(Tice et al 2008). This kNN/BCSC model was then tested on two French screening pops after adjustment on French BC incidence: an urban area (Paris suburbs, N=316 775) and a rural area (Côte d'Or, N=32 930). Its performances were compared to those of a model directly developed (same methods) on the Paris cohort (kNN/Paris). Levels of individual risks assessed by the models were assigned into 4 risk categories. The sensitivity of the models was defined as the number of wn who had BC whose 5 yrs-risk category was intermediate (median risk at 50-yrs - 1.66%), high (> 1.66%) or very high (> 20% lifetime) divided by the total number of wn who had BC.

Results: The performances of the different models are shown in Table 1. The kNN model developed on BCSC performed well (c-stat 0.653 and E/O 1.001). It had equivalent performances as the parametric model developed previously on the same pop. This kNN/BCSC had a good discrimination on French pops, although slightly lower than that on US pops. This is expected since French screening starts at 50 (vs 40 in BCSC) and French parameters do not include race. The calibration of such model was excellent on Paris, while it overestimated the risk on Côte d'Or, in which BC incidence is lower. It performed as well as the kNN/Paris model directly developed on Paris' pop. The sensitivity of the kNN/BCSC on US and French pops was good.

Conclusions: A new non parametric kNN breast cancer risk model developed on an American screening cohort (BCSC) was successfully validated on two French screening cohorts. This new international model could allow stratified prevention.

Performances of the models

	Param/BCSC on BCSC	Param/BCSC on Paris	kNN/BCSC on BCSC	kNN/BCSC on Paris	kNN/BCSC on Cote d'Or	kNN/Paris on Paris
N	629 229	313 817	629 229	313 817	32 930	313 817
c-statistic	0.658	0.602	0.653	0.602	0.593	0.605
E/O global	1.03	1.07	1.001	1.06	1.52	1.00
Sensitivity	-	-	76.18%	72.87%	72.58%	-

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-06-06

Title: A clinical model for assessment of the individual breast cancer risk

Eriksson M, Czene K, Pawitan Y, Leifland K, Darabi H and Hall P. Karolinska Institutet, Stockholm, Sweden and South General Hospital, Stockholm, Sweden.

Body: Background. Most mammography screening programs are not individualized. To efficiently screen for breast cancer the individual risk of the disease should be determined. We describe a model that estimate the 2-year risk of breast cancer and could be used at most mammography screening units without adding substantial cost.

Methods. The study was based on the population based prospective Karma cohort including 70,877 participants. Mammograms were collected up to three years following baseline mammogram. A prediction protocol was developed using mammographic features (density, microcalcifications and masses), use of hormone replacement therapy (HRT), family history of breast cancer, menopausal status, age, and body mass index. Relative risks were calculated using conditional logistic regression. Absolute risks were calculated using the iCARE protocol.

Results. Comparing women at highest and lowest mammographic density yielded a 5-fold higher risk of breast cancer for women at highest density. When adding microcalcifications and masses to the model, high-risk women had a nearly 9-fold higher risk of breast cancer compared to those at lowest risk. The difference in microcalcifications and masses between left and right breast was a better predictor of breast cancer than number of microcalcifications and masses in the breasts.

When calculating the absolute 2-year risk of breast cancer we stratified women using the NICE guidelines for 10-year risk divided by 5 (**Table 1**). The mean absolute 2-year risk of breast cancer in the different risk categories was 0.12%, 0.33%, 0.83% and 1.95% for women at low, moderate, general, and high risk. In most countries with established mammography screening programs approximately 5 women in a 1000 are diagnosed with breast cancer at regular screening. We managed to identify a low risk group of approximately 10% of all women where 1 woman in a 1000 will be diagnosed with breast cancer, contrasting the 2% of all women at highest risk where 20 women out of a 1000 will be diagnosed with breast cancer within 2 years of last negative screen (**Table 1**).

In the full model, taking HRT use, family history of breast cancer and menopausal status into consideration, area under the curve (AUC) reached 0.71.

Conclusions. Our model includes three mammographic features that could easily be derived from clinically available software. By adding information on some few established risk factors it is possible to improve clinical care by identifying women in need of additional examination procedures. At the same time there is a substantial proportion of women that will have very little benefit from mammography screening due to their low risk of breast cancer.

Table 1. Absolute 2-year risk of breast cancer in women stratified in to risk categories based on the NICE guidelines

Absolute 2-year risk (risk group)	Percent women at risk	Mean absolute 2-year risk ²	Stratified 2-year risk
0-0.15 (low)	10.3	0.12	1.0 (reference)
0.15-<0.6 (general)	64.8	0.33	2.75
0.6-<1.6 (moderate)	22.9	0.82	6.83
≥1.6 (high)	2.0	1.95	16.2

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-06-07

Title: Risk of metastases after ipsilateral breast tumour recurrence changes overtime according to patient and tumour characteristics: Implications for treatment

Gennaro M, Di Cosimo S, Ardoino I, Veneroni S, Mariani L, de Braud F, Daidone MG, Biganzoli E and Demicheli R. Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy.

Body: To evaluate the risk of systemic disease of early breast cancer (EBC) patients undergoing breast conservative surgery between 1970 - 2000 and experiencing ipsilateral breast tumour recurrence (IBTR). **Patients and Methods** The study population included EBC patients randomly assigned onto 5 Milan prospective trials (n= 915) or comparably treated outside clinical trials (n= 1060) with quadrantectomy followed by radiotherapy and with known primary tumor characteristics. Multivariable regression analysis was performed via the piecewise exponential. The model accounts other than known prognostic factors, including age, menopausal status, pathologic size, nodal status and time dependent effect for estrogen receptor (ER) for the IBTR occurrence as time dependent covariate (i.e. switching from 0 to 1 at the time of surgery for IBTR). Also the new time scale induced by IBTR occurrence, namely time elapsed since surgery for IBTR to the end-point of interest, was accounted for in the model.

Results Median follow-up was 124 months (IQ Range 96.6 – 179). At the time of analysis, 504 patients were died, 470 presented with systemic disease and 150 with IBTR as first breast cancer event. IBTR was associated with a high risk of systemic disease. This risk was dependent on primary tumor characteristics, including T size, 2 *versus* 1 (HR 1.79 95% CI 1.46 – 2.21), and nodal status, N1 *versus* N0 (HR 1.61, 95% CI 1.32 – 1.98), and N2 *versus* N0 (HR 3.91, 95% CI 3.13 – 4.89). Of note, ER-negative cohort had a slightly earlier and higher hazard of events overtime compared with ER-positive cohort, which, instead, presented higher hazard estimates over the long follow-up period. The most important finding concerned the new timescale induced by IBTR occurrence that revealed to have definite prognostic value, and suggest that patients with IBTR as compared with patients with no IBTR have a sudden and at least 5-fold increase of risk of systemic disease within 2 years following second surgery; of note, this risk decreases thereafter, though it never disappears. **Conclusion** Breast cancer patients experiencing IBTR are at increased risk of systemic disease. This risk changes overtime and is different according to initial patient and primary tumor characteristics, and hormonal receptor status. The definition of different patterns of BC recurrence can improve BC care through surveillance guidelines and can guide the design of tailored clinical studies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-06-08

Title: Race is not a contributing factor to breast cancer-free interval outcome for patients with equal access to healthcare treated at a military medical center

Liu J, Fantacone_Campbell JL L, Kovatich AJ J, Hooke JA A, Kvecher L, Sturtz LA A, Shriver CD D and Hu H. Windber Research Institute, Windber, PA; Clinical Breast Care Project, Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD and Walter Reed National Military Medical Center, Bethesda, MD.

Body: BACKGROUND Black patients diagnosed with invasive breast cancer (IBC) are more likely to die of the disease than their White counterparts. Differences in IBC subtype, socioeconomic status, and access to healthcare may be contributing factors. We performed this study to determine if there is a racial disparity in breast cancer-free interval (BCFI) for patients treated at a military medical center where equal-access healthcare is provided.

METHODS Three datasets were used: 1) TCGA-Breast Cancer cohort with 149 Black and 706 White patients; 2) Clinical Breast Care Project patients from a military medical center (CBCP-WR) with 112 Black and 372 White patients; and 3) CBCP patients from a civilian facility (CBCP-AAMC) without equal-access healthcare (data collection is ongoing and results will be available for the conference). IHC of ER, PR, and HER2 was performed to subtype IBCs as TN (ER-/PR-/HER2-) or Non-TN. Patients with Stage IV IBC at diagnosis were excluded. Kaplan-Meier method was used to generate survival plots. Cox proportional hazards regression model was used for survival analysis between races (Black vs. White). Univariable and multivariable analyses were performed. All analyses were performed using R 3.2.2. A two-tailed p value < 0.05 was considered significant.

RESULTS The clinicopathologic properties of Black and White patients in TCGA and CBCP-WR cohorts were analyzed, and the results are consistent with the literature. The median follow-up times were comparable between the two races (TCGA: Black=29.4 M, White=27.5 M; CBCP-WR: Black=61.1 M, White=60.9 M).

In TCGA, race significantly affects BCFI in multivariable analysis after adjustment for age, AJCC stage, and PAM50 subtype (HR=1.73, 95%CI=1.02-2.94, p=0.042). Stratifying patients into TN (Black=46.7%; White=53.3%) and Non-TN (Black=14.2%; White=85.8%) subtypes, race trended towards significant in BCFI in TN (HR=2.33, 95%CI=0.98-5.56, p=0.052) but not Non-TN subtypes (HR=1.12, 95%CI=0.55-2.27, p=0.752). Further adjustment for age and AJCC stage did not alter the results.

In CBCP-WR, there was no significant racial difference in both univariable (HR=1.52, 95%CI=0.87-2.68, p=0.145) and multivariable analysis of BCFI adjusted for age, subtype and AJCC stage (HR=1.00, 95%CI=0.5-2.01, p=0.989). Stratifying patients into TN (Black=43.5%; White=56.5%) and Non-TN (Black=18.8%; White=91.2%) subtypes, race was not a significant factor in either group (TN: HR=0.920, 95%CI=0.4-2.14, p=0.846; Non-TN: HR=1.17, 95%CI=0.53-2.61, p=0.701). Further adjustment for age and AJCC stage did not alter the results.

CONCLUSIONS Our results show that in the CBCP-WR cohort, there is no racial disparity in BCFI at the whole cohort level nor is there any trend in the TN subtype. These results contradict those obtained from the TCGA cohort suggesting unequal healthcare access may be a major contributor to racial disparity in BCFI. Analysis of the CBCP-AAMC cohort may provide further insight into whether treatment at a single medical center needs to be considered when investigating racial disparities in BCFI.

The views expressed in this article are those of the author and do not reflect the official policy of the Department of Defense, or U.S. Government.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-06-09

Title: Prediction of relapse in patients with locally advanced breast cancer after neoadjuvant treatment

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Body: BACKGROUND. Despite advances in cancer treatment, over 25% of patients (pts) with locally advanced breast cancer (LABC) relapse (DR) during first 5 years after treatment (Trmt).

OBJECTIVES. The primary objective was to construct a prediction tool for risk of relapse (RoR) in pts with LABC after neoadjuvant therapy (NAT). Previously published works (Matsuda N. et al, 2014; Kearn B. et al, 2011; Katz A. et al. 2008) have also examined this issue.

MATERIAL AND METHODS. This was single center, retrospective study of 546 pts with LABC who received NAT at the Ottawa Hospital Cancer Center between 2005 and 2015. Median follow-up (FU) was 49 months. The following data collected: demographics, tumor size, nodal and receptor status, grade, HER-2, stage of disease, cancer Trmt and clinical outcomes. Primary endpoints were local (L) and/or distant (D) DR rate during first 5 years and time to DR during the first 5 years. A prediction tool was devised based on the Cox regression model.

RESULTS. In 545 pts NAT was prescribed as follows: FEC-D – 91 (17%), AC-Docetaxel – 330 (60%), other regimens (AC, AC-Paclitaxel, TC, TCH)– 124 (23%). Breast conserving surgery was performed in 67 (12%) pts, mastectomy in 440 (81%) pts. Adjuvant radiotherapy was given in 485 (89%). All patients had trastuzumab – 173 pts (34%) for Her2-positive disease and endocrine Trmt (tamoxifen and/or AI) – 356 (44%) pts – for endocrine-sensitive disease. DR rate during first 5 years of FU was 17.3% (L DR – 3.2%, D DR – 13.2%, L+D DR – 0.9%).

Over 60 variables were included in primary analysis. Cox regression proportional hazards model resulted in only 5 factors with significant influence on RoR during first 5 years of FU. Risk factors and their risk prediction value are: 1) residual disease (yes- 4; no-0), (HR = 4.25; p-value=0.000), 2) lymph nodes status (positive-3; negative-0), (HR = 2.27; p-value=0.006), 3) Inflammatory histology (yes-2; no-0), (HR = 1.90; p-value=0.003) 4) estrogen receptors status (positive-2; negative-0), (HR = 2.07; p-value=0.001), 5) Adjuvant radiotherapy (yes-0; no-1), (HR = 1.76; p-value=0.036). When these factors are combined the following Relapse Prediction (RP) Score can be constructed (table 1).

Table 1. Risk prediction score

Score	Risk of relapse (5 years)	No of patients	No of pts with DR
0-5	Low – 7%	153 (28%) Censored (C): 77 Analysed (A): 76	L:3 (4%) D:2(3%) L+D:0
6-7	Intermediate – 26%	220 (40%) C: 96 A: 124	L:5 (4%) D:27 (22%) L+D:0
8-12	High – 51%	172 (32%) C: 59 A: 113	L:9 (8%) D:43 (38%) L+D:5 (4.5%)

Internal validation of proposed model was performed. ROC analysis revealed a sensitivity of 75%. According to this simple RP score, pts can be classified into to three groups (RP score – 0-5; 6-7; 8-12). RoR was 7 times higher in patients with RP Score 8-12 vs pts with score 0-5 (p-value<0.0001).

CONCLUSIONS. Pts with LABC represent a heterogeneous group with diverse risk of DR. Our prognostic tool based on 5 risk factors can be used to predict RoR after NAT with a sensitivity of 75%. Pts with high risk may require additional Trmt and/or more active FU strategies and this simple model may be used to design unique studies in LABC based on RP score. We intend to further validate this model on a larger multi center /provincial population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-01

Title: Chemoprevention uptake among women with atypical hyperplasia, lobular and ductal carcinoma *in situ*

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Body: Introduction: Chemoprevention with anti-estrogens can reduce breast cancer risk among high-risk women. However, uptake is estimated to be lower than 15% among women offered anti-estrogens. Women with atypical hyperplasia (AH), lobular carcinoma *in situ* (LCIS), and ductal carcinoma *in situ* (DCIS) are at an increased risk of developing invasive breast cancer and often derive more benefit from anti-estrogens compared to other high-risk populations. We sought to determine which factors are associated with chemoprevention uptake in a population of women with AH, LCIS, and DCIS.

Methods: We conducted a retrospective cohort study at an urban academic center in New York, NY of women diagnosed with AH/LCIS/DCIS between 2007 and 2015 without a history of invasive breast cancer (n=1719). Demographic and clinical information, including type of anti-estrogen and medical oncology referral, were collected from the electronic health record. Breast disease in each patient was classified according to the most advanced lesion (DCIS>LCIS>AH). A subset of women with AH/LCIS/DCIS scheduled for an initial consultation with a medical oncologist (n=73) completed questionnaires on their breast cancer and chemoprevention knowledge, risk perception, and behavioral intentions. Descriptive statistics were generated and univariate and multivariable log-binomial regression were used to estimate the association between sociodemographic and clinical factors and chemoprevention uptake.

Results: In our sample, mean age was 60 years (SD 12); white/black/Hispanic/Asian/other (%): 45/9/23/6/17; AH/LCIS/DCIS (%): 35/24/41; and 33% were referred to a medical oncologist. A total of 505 (29%) women had initiated an anti-estrogen, including 54% who used tamoxifen, 15% raloxifene, 19% aromatase inhibitors, and 11% who tried multiple anti-estrogens. Older women and Hispanics compared to non-Hispanic whites were more likely to take anti-estrogens. Compared to women with AH, LCIS (RR: 1.43; 95% CI: 1.16-1.76) and DCIS (RR: 1.54; 95% CI: 1.28-1.86) were significantly associated with chemoprevention uptake. Medical oncology referral was the strongest predictor of chemoprevention uptake (RR: 5.79; 95% CI: 4.80-6.98). According to the survey data, many women had heard of anti-estrogens for chemoprevention (75%), but few were knowledgeable about it. The majority of participants were worried about the side effects of chemoprevention (72%) and considered them very serious (57%). Satisfaction was high among those who reported making a decision to take chemoprevention, however, only 50% of survey participants thought the benefits of anti-estrogens were worth the risks.

Conclusions: At our center, women with AH, LCIS, and DCIS have higher rates of chemoprevention uptake compared to the reported literature. Despite the potential for younger women to see a greater lifelong benefit from chemoprevention, our results indicate this population may be less likely to use anti-estrogens. Misperceptions about personal breast cancer risk and chemoprevention adverse effects may be barriers to uptake. Improving patient-provider communication about breast cancer risk and the risks and benefits of chemoprevention may facilitate informed-decision making about anti-estrogen therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-02

Title: Concordance with National comprehensive cancer network (NCCN) metastatic breast cancer guidelines and impact on overall survival

Rocque GB B, Williams CP P, Jackson BE E, Halilova KI I, Pisu M, Andres F and Smita B. University of Alabama at Birmingham, Birmingham, AL.

Body: Introduction: Payers are implementing reimbursement restrictions for non-guideline based care. Limited information exists regarding real-world concordance with guidelines for metastatic breast cancer (MBC) treatment. Further, the impact of non-concordance on mortality is unknown. We address these gaps by using the Surveillance, Epidemiology, and End Results (SEER) Program-linked Medicare database to evaluate national concordance with NCCN guidelines and the association between concordance and mortality.

Methods: From 2007 to 2013, women with *de novo* (n=988) or recurrent metastatic breast cancer (n=5651) were evaluated for concordance of first-line systemic therapy (hormonal therapy, chemotherapy, and Her2-targeted therapy) with NCCN guidelines. Concordance was defined as receipt of single agent or combination treatments listed on NCCN guidelines. Non-concordant treatments were grouped into 5 categories: single-agent HER2-targeted therapy (33%), adjuvant regimens used in the metastatic setting (12%), therapy mismatched with ER/HER2 status (12%), non-approved bevacizumab regimens (10%), and other miscellaneous reasons (33%). Multivariable logistic regression was used to identify predictors of non-concordance. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated using Cox regression to compare all-cause mortality associated with non-concordant vs. concordant treatment adjusted for receptor status, comorbidities, age, race, poverty level, entitlement reason, and treatment year.

Results: Mean age at MBC diagnosis was 69y; 77% were white. Median follow-up was 1.2 years. The prevalence of non-concordant first-line systemic therapy was 19% for *de novo* MBC and 18% for recurrent MBC. Younger age, hormone-receptor negative status, and Her2-positive status were associated with non-concordant treatments for Stage IV and recurrent MBC patients ($p < 0.001$). Higher poverty by census tract was associated with non-concordance in recurrent MBC ($p < 0.05$). The most frequent category of non-concordant treatment in *de novo* MBC was use of adjuvant regimens in Stage IV MBC (43%) and use of single-agent HER2-targeted therapy (31%) in recurrent MBCs. Adjusted overall survival was similar for patients with *de novo* MBC receiving concordant and non-concordant treatments (HR 0.88, CI 0.72-1.65). Mortality was modestly increased for patients with recurrent MBC receiving non-concordant care (HR 1.12, CI 1.02-1.22); however, substantial differences were noted by category of non-concordance. Compared to concordant treatment, single-agent HER2-targeted therapy was associated with decreased risk of mortality (HR 0.78, CI 0.68-0.91). Increased mortality was observed for non-approved bevacizumab use (HR 1.79, CI 1.44-2.22) and other miscellaneous regimens (HR 1.42, CI 1.26-1.60). Mortality for therapy mismatched with ER/HER2 status was similar to concordant treatment (HR 1.13, CI 0.88-1.44).

Conclusions: In the first-line setting, treatment inconsistent with NCCN guidelines remains common (18%). Overall mortality was not substantially higher among non-concordant patients. However, mortality risk varied (in both directions) by category of non-concordance. These findings may provide an opportunity for considering refinement of NCCN guidelines.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-03

Title: Insulin and breast cancer risk: Novel insights from mammographic density analyses

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Body: Background: Insulin has been suspected to influence breast cancer risk because of its mitogenic effects and impact on sex hormone levels. Epidemiological studies investigating breast cancer occurrence in insulin-treated patients with diabetes have produced conflicting results, but were often underpowered and lacked adequate control for important confounders.

Purpose: To investigate the impact of insulin treatment on mammographic density (MD) as intermediate phenotype for breast cancer risk, and to further explore causation by analyzing associations with a polygenic risk score (PRS) incorporating 18 single nucleotide polymorphisms (SNPs) associated with fasting insulin levels.

Design: We conducted a matched cohort study within a Swedish screening-based cohort including insulin-treated type 1 (T1D, N=122) and type 2 (T2D, N=237) diabetes patients and up to 5 non-diabetic women matched on birth year (N=1,780).

Associations between diabetes status and duration of insulin use with volumetric mammographic density were analyzed using general linear models adjusting for a comprehensive set of potential confounders. PRS analyses were performed in an independent sample of non-diabetic women (N=9,437) from the same cohort.

Results: In multivariable analyses, T1D patients had higher percent (11.2 vs. 8.8%; $P<0.001$) and absolute dense volumes (66.6 vs. 60.8 cm³; $P=0.09$) and a lower absolute non-dense volume (513.2 vs. 617.7 cm³; $P<0.001$) compared to age-matched non-diabetics. Percent- and absolute dense volumes increased with increasing T1D duration, while no such trend was observed for the absolute non-dense volume. Similar associations but of smaller magnitude, were found in insulin-treated T2D patients. Genetically predicted higher fasting insulin levels among non-diabetes women were associated with increased percent and absolute dense volumes (% change per sd increase in PRS = 0.8 (0.0-1.6) and 0.9 (0.1-1.8) respectively), but no difference in absolute non-dense volume.

Conclusions: Our results demonstrate higher mammographic density among insulin-treated diabetes patients, and genetic analyses support an effect of insulin on volumetric mammographic density. Further studies into how the observed MD differences translate into breast cancer risk are warranted.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-04

Title: Risk of primary and contralateral breast cancer in BRCA1/2 mutation carriers previously affected with ovarian cancer

Heemskerk-Gerritsen BAM AM, Hooning MJ J, van Doorn HC C, Collée MJ J, Koppert LB B, Jager A, van den Ouweland AMW MW, Netherlands H, Seynaeve C and Kriege M. Erasmus MC Cancer Institute, Rotterdam, Netherlands and Hereditary Breast and Ovarian Cancer Cohort Study, Netherlands.

Body: Introduction: BRCA1/2 mutation carriers are at increased risk of developing breast cancer (BC) and ovarian cancer (OC). However, for BRCA1/2 mutation carriers previously affected with OC, primary BC (PBC) and contralateral BC (CBC) risks might be lowered due to OC treatment, but data hereon are limited. Accurate BC risk estimates are essential for counseling and optimal decision making on BC surveillance and risk-reducing mastectomy (RRM) after diagnosis of BRCA1/2-associated OC.

Methods: From the national HEBON cohort study, we selected female BRCA1/2 mutation carriers born from 1940 and at risk of PBC (n=2019; 1194 BRCA1, 825 BRCA2) or at risk of CBC (n=1276; 839 BRCA1, 437 BRCA2). Eligibility criteria for the PBC risk analyses included no history of PBC and both breasts in situ at DNA testing. For the CBC risk analyses, CBC before DNA testing was not allowed and the contralateral breast had to be unaffected at DNA testing. To estimate the association between OC and BC risk, we used the Fine and Gray regression model, with OC as a time-dependent variable and death as competing risk event. The observation started at the age of DNA testing, age 35, or – for the CBC risk analyses – age at PBC diagnosis, whichever came last. Censoring events were RRM, study closing date (i.e. 2014-12-31), and – for the CBC risk analyses – ipsilateral BC recurrence. All analyses were performed separately for BRCA1 and BRCA2 mutation carriers.

Results: For the PBC analyses, we selected 236 BRCA1/2 mutation carriers with OC and 1783 BRCA1/2 mutation carriers without OC (median age at start study 53 and 41 years, respectively). During a mean follow-up (FU) of 5.2 years, 15 (6%) OC patients developed PBC after OC, while PBC was diagnosed in 262 (15%) women without OC. OC diagnosis was associated with a lower PBC risk in BRCA1 mutation carriers (HR, 0.27; 95% CI, 0.14–0.53) but not in BRCA2 mutation carriers (HR, 0.88; 95% CI, 0.34–2.27). The cumulative PBC incidence at age 70 in BRCA1 mutation carriers was 25% for OC patients and 66% for women without OC, and in BRCA2 mutation carriers 49% for OC patients and 53% for women without OC.

For the CBC analyses, we selected 99 BRCA1/2 mutation carriers affected with PBC and OC, and 1177 BRCA1/2-associated PBC patients without OC (median age at start study 54 and 46 years, respectively). During a mean FU of 3.5 years, 8 (8%) PBC/OC patients developed CBC after OC, while CBC was diagnosed in 131 (11%) PBC patients without OC. In BRCA1 mutation carriers, OC diagnosis was non-significantly associated with a lower CBC risk (HR, 0.44; 95% CI, 0.19–1.03). For BRCA2 mutation carriers the HR was 1.65 (95% CI, 0.34–8.04). The cumulative CBC incidence at age 70 in BRCA1 mutation carriers was 40% for OC patients and 68% for patients without OC; for BRCA2 mutation carriers the number of CBCs was too small to obtain meaningful estimates.

Conclusion:– For BRCA1 mutation carriers affected with OC the risk of developing subsequent BC is lower than for BRCA1 mutation carriers without OC. Our data suggest that the current BC prevention strategies should be reconsidered for OC patients carrying a BRCA1 mutation, possibly including less intensive BC surveillance, and RRM being indicated only for a subgroup of OC patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-05

Title: Risk of metachronous contralateral breast cancer: Systematic review and meta-analysis

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Body: Introduction

Over the last 2 decades, an increasing number of primary breast cancer (PBC) patients opted for a risk reducing contralateral mastectomy, to minimize risk of subsequent contralateral breast cancer (CBC). Therefore, accurate risk estimates of CBC are important for patient tailored counseling and decision making regarding treatment. Currently, CBC risk estimates are determined by *BRCA1/2* mutation status, age at PBC diagnosis and family history. For other risk factors, results are inconclusive.

We therefore aimed to quantify the association with CBC risk for patient, tumor and treatment related factors as reported in the literature.

Methods

Medline was searched for publications on CBC risk by one reviewer (DA). We focused on associations between CBC risk and *BRCA1/2* and *CHEK2*1100delC* mutations, SNPs, risk-reducing salpingo-oophorectomy and various factors at PBC diagnosis: family history of breast cancer (BC), age, BMI, menopausal status, mammographic density, TNM-stage, receptor status, morphology, administered radiotherapy and adjuvant systemic treatment.

Eligible papers were published in English between 01-01-1990 and 01-04-2015, investigated female patients with invasive early BC and reported relative risk (RR) estimates (i.e., hazard ratios, relative risks or odds ratios).

We combined RR estimates using a random effects model. Heterogeneity was assessed using the I^2 statistic. Forest plots for crude and adjusted estimates were generated stratifying for mutation status (i.e., *BRCA1*, *BRCA2*, *CHEK2*1100delC*), non-carriers and unselected patients (i.e., population/hospital based cohorts).

Results

After screening of 1,423 papers for title and abstract, 173 eligible papers were fully read, and 96 papers fulfilled the inclusion criteria.

Both in the unselected group and in the *BRCA1* and *BRCA2* groups, administration of adjuvant endocrine therapy (vs. not), was associated with decreased CBC risk (RR, 0.62 (95% CI 0.55-0.69), 0.55 (95% CI 0.39-0.77), and 0.62 (95% CI 0.40-0.95), respectively). Adjuvant chemotherapy was associated with reduced CBC risk in unselected patients (RR 0.73; 95% CI 0.62-0.86). CBC risk was increased in unselected patients who received radiotherapy at age <40 years (vs. not) (RR 1.33; 95% CI 1.18-1.49), had a lobular (vs. ductal) PBC (RR 1.70; 95% CI 1.33-2.16), had a T2/T3 (vs. T1) PBC (RR 1.15; 95% CI 1.01-1.30), with BMI ≥ 30 (vs. <25) at PBC (RR 1.50; 95% CI 1.27-1.76) or had a positive family history for BC (vs. not) (RR 1.82; 95% CI 1.35-2.46). For the other factors of interest we did not observe any effects on CBC risk.

For *CHEK2*1100delC* mutation carriers and non-carriers, information on factors affecting CBC risk was scarce. In non-carriers, the RR for adjuvant chemotherapy was 0.60 (95% CI 0.53-0.68).

Conclusions

In unselected patients, adjuvant systemic treatment for PBC decreases CBC risk while a lobular morphology of PBC, high BMI, and a family history of BC increase the risk of CBC. Data is scarce for carriers of a *BRCA1/2* or *CHEK2*1100delC* mutation and non-carriers. This review identifies prognostic factors to consider for individualized CBC risk estimation which may support medical decision making in BC patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-06

Title: Obesity, dyslipidemia, and diabetes as risk factors for trastuzumab-related cardiotoxicity in breast cancer patients

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Body: Background. Clinical trials have demonstrated an increased risk of cancer treatment related cardiac dysfunction (CTCD) during trastuzumab(H) therapy. Diabetes, dyslipidemia, and obesity are also known risk factors for cardiovascular disease. The primary objective of this study was to evaluate the incidence of asymptomatic drop in left ventricular ejection fraction (AD-LVEF) and symptomatic CTCD (S-CTCD) in women with BC and co-morbidities receiving H, referred to a dedicated cardio-oncology clinic (COC).

Methods. This was a retrospective cohort study of women with BC (all stages) referred to The Ottawa Hospital COC between 2008 and 2015, who received chemotherapy (CT) and H. Data collected included: demographics, reason for referral, cardiac testing (MUGA/ECHO) co-morbidities (diabetes, dyslipidemia) and cardiac outcomes. Obesity defined as body mass index > 30. Symptomatic CTCD include SOB, PND, orthopnea, peripheral edema, congestive heart failure. Asymptomatic drop in LVEF defined as decrease of at least 15% from baseline; normal LVEF, or a decrease of at least 10% in LVEF, less than 53%.

Results. Chemotherapy (A-CT) followed by H, 62 received H without A-CT. 139 patients with no history diabetes, dyslipidemia, or obesity, referred with AD-LVEF (68%), with S-CTCD (30%), pre-CT assessment (2%). Obesity (O) pre-existing co-morbidity in 63 (26%) patients, (20 (32%) with S-CTCD, 40 (63%) referred with AD-LVEF, 3(5%) referred for pre-CT assessment). Dyslipidemia (L) – 52 (21%); referred with S-CTCD 15; 29%), with AD-LVEF 31 (60%), pre-CT assessment 6 (11). Diabetes (D) – 29 (12%) - (6 (21%) with S-CTCD, 19 (66%) referred with AD-LVEF, 4(13%) referred for pre-CT assessment). Combination of two or three conditions significantly increase incidence of S-CTCD: O+L – 67%, O+D – 69%, O+L+D – 72%. Combination of obesity and other conditions significantly increase incidence of S-CTCD: O+L – 67%(Relative Risk -2.2, p=0,04), O+D – 69% (RR-2.3, p=0,02), O+L+D – 72% (RR-2.4, p=0,08).

Conclusion. The combination of two or three co-morbidities significantly increases the incidence of S-CTCD. BC patients experiencing CTCD with pre-existing history of diabetes, dyslipidemia, and obesity may require more proactive strategies for prevention, detection and treatment of cardiotoxicity during trastuzumab-based treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-07

Title: Comparison of survival and clinicopathological features between patients with breast cancer and HIV infection versus breast cancer without HIV infection

Gligich O, Acuna A, Khan H, Kim R, Wang D, Sparano J and Anampa J. Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY.

Body: Background:

Breast cancer (BCA) is the most common malignancy and the most common cause of death among females worldwide. The use of antiretroviral therapy prolonged life expectancy and increased the incidence of age-related malignancies in patients with the human immunodeficiency virus (HIV). Data regarding the protective versus deleterious effect of HIV in the presentation, clinical course and mortality of patients with BCA is limited.

Objective: To compare the median overall survival between patients with and without HIV diagnosed with breast cancer between 2000 and 2014 at Montefiore Medical Center.

Methods: Patients with HIV infection and breast cancer diagnosed between 2000 and 2014 were identified. Controls were randomly selected from a pool of 5552 patients with BCA without HIV using stratified sampling by year of breast cancer diagnosis with a ratio of 3 controls per case. Patients were followed up for a median of 52 months. Median overall survival and clinicopathological characteristics were compared among groups.

Results: A total of 42 cases of BCS with HIV infection were identified. Of 126 selected controls; 15 were excluded after chart review. Patients with HIV were diagnosed with breast cancer at significantly younger ages compared to non-HIV patient (50.6 vs 64.8, $P < .001$), had lower BMI (26 vs 30.1, $p < 0.001$) and had higher frequency of Not-hispanic Black (NHB) (64.3% vs 41.4%). Breast cancer stage, grade, histological type and hormonal status as well as surgery, chemotherapy and hormonal therapy were not significantly different between the groups; but the HIV group had a tendency towards less hormonal therapy. There was no difference in the rate of mortality; however overall median survival was higher in patient with HIV. This difference was not statistically significant (106 vs 72, $p = 0.78$)

Conclusion: Patients with HIV are diagnosed with breast cancer at younger ages than the general population. Closer monitoring in this population was suggested by other authors; however the rate of stage I disease is similar between both groups. Despite similar rates of surgery and chemotherapy, higher rates of NHB and less hormonal therapy, mortality rates were similar in both groups and median overall survival was higher in patient with HIV.

	Breast cancer with HIV (n = 42)	Breast cancer without HIV (n = 111)	p-value*
Age at BCA diagnosis	50.6 (11.2)	64.8 (14.4)	< 0.001
BMI	26.0 (6.2)	30.1 (7.4)	< 0.001
Race, n (%)			0.014
White, Not-Hispanic	3 (7.1)	23 (20.7)	
Black, Not-Hispanic	27 (64.3)	46 (41.4)	
Hispanic	9 (21.4)	28 (25.2)	
Other/unknown	3 (7.1)	14 (12.6)	
Post menopausal, yes (%)	16 (40)	84 (80.7)	< 0.001
Breast cancer stage, n (%)			0.15
1	15 (37.5)	39 (39.00)	
2	15 (37.5)	43 (43.00)	
3	8 (20)	18 (18.00)	
4	2 (5.00)	0 (0.00)	
Breast cancer grade, poor (%)	17 (44.7)	42 (42.4)	0.94

Histological type, n (%)			0.10
IDC	39 (92.86)	89 (80.18)	
ILC	2 (4.76)	6 (5.41)	
Mixed	1 (2.38)	16 (14.41)	
Estrogen receptor, pos (%)	25 (65.8)	69 (72.6)	0.43
Progesterone receptor, pos (%)	20 (52.6)	52 (55.9)	0.73
Her 2 status, pos (%)	11 (29)	15 (16.5)	0.11
Chemotherapy, n (%)			0.45
1	6 (15.38)	13 (14.29)	
2	22 (56.41)	39 (42.86)	
3	1 (2.56)	3 (3.30)	
4	10 (25.64)	36 (39.56)	
Hormonal therapy, yes (%)	21 (53.9)	54 (70.1)	0.08
Dead, n (%)	20 (48)	57 (51)	0.68
Recurrence, n (%)	9 (21)	27 (24)	0.71
Median overall-survival in mo	106	72	0.78**

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-08

Title: Neuroleptics in breast cancer risk

Johnston A, Garcia S, Hein S, Bu W and Yi L. Baylor College of Medicine, Houston, TX.

Body: Psychiatric medications are among the most widely prescribed class of drugs in the U.S.; the main mechanism of action for a majority of both class 1 and 2 of antipsychotics is blockade of the dopamine receptors in the brain. Dopamine signaling blocks the release of prolactin in the hypothalamus; thus, a common side-effect of anti-psychotic medications is hyperprolactinemia, which causes breast swelling and lactation unrelated to a pregnancy. Elevated serum prolactin levels are associated with increased breast cancer risk. Prolactin-stimulated PRLR/JAK/STAT signaling suppresses cell apoptosis and promotes mammary early lesion progression to cancer. However, it is not yet known whether these dopamine receptor-blocking antipsychotics increase breast cancer risk, and evidence-based research on this topic remains limited. We found that treatment of mice bearing precancerous early lesions with either pimozide (a class 1 antipsychotic) or risperidone (a more commonly prescribed class 2 antipsychotic) caused hyperprolactinemia, activated STAT5 and, unexpectedly, STAT3 in these early lesions, suppressed cell apoptosis, and increased the early lesion load. Furthermore, we observed that continual risperidone treatment, which mimics the treatment scheme of a great majority of patients on this type of medication, accelerated the progression of these early lesions to cancer. Additionally, we found that a short-course of ruxolitinib, an FDA-approved JAK1/2 inhibitor, prevented the cancer-promoting effects of risperidone. Collectively, these data suggest that hyperprolactinaemia-inducing antipsychotics lower the apoptotic anticancer barrier in precancerous early lesions and increases breast cancer risk, and prophylactic treatment with ruxolitinib can lower the risk of breast cancer in women on these neuroleptics. RRPA and other genetic experiments have led to potential mechanisms underlying the weakened apoptotic anticancer barrier in these antipsychotic-treated mice.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-09

Title: CPM rate among individuals with breast cancer who underwent multiplex gene testing for hereditary cancer: Single institution experience

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Body: Background: Availability of multiplex gene (MPG) testing for hereditary cancer has led to increase use of panel testing versus single gene testing for hereditary cancer. These panels include high, but also moderate penetrance genes. For some of these genes, associated cancer risk and risk management guidelines do not exist. Furthermore there is a high rate of variant of unknown significant (VUS) findings in non-BRCA genes. Currently, in the absence or minimal available data, health care providers and patients are faced with important risk management decisions, especially regarding preventive surgeries, such as prophylactic mastectomy. Currently, there is no data regarding prophylactic mastectomy rate among patients with breast cancer who underwent (MPG) testing. Therefore, our aim was to evaluate the rate of contralateral prophylactic mastectomy (CPM) in a cohort of individuals who underwent multiplex gene testing. **Methods:** Eight hundred thirty five patients with breast cancer who underwent MPG testing between the years 2013 and 2016 were identified using Institutional Clinical Cancer Genetics Database. Patients with pathogenic, likely pathogenic variants or who had a VUS were included in the analysis. **Results:** Of 835 patients with a diagnosis of breast cancer; 105 (13%) had a pathogenic or likely pathogenic mutation: 29 (28%) BRCA1, 26 (25%) BRCA2, 11 (11%) ATM, 2 (2%) BARD1, 3 (3%) BRIP, 9 (9%) CHEK2, 1 (1%) MSH2, 1 (1%) NBN, 5 (5%) PALB2, 4 (4%) PTEN, 1 (1%) RAD51C, 5 (5%) TP53, 4 (4%) CDH1, 2 (2%) MUTYH, 1 (1%) PMS2, 1 (1%) APC (1). A total of 102 (12%) VUS were found. Average age of diagnosis was 44 (Range 21-81). CPM rate was 32% (n=66) for the total cohort. Twenty nine % (n=19) of patients with non-BRCA mutations, 24.2% (n=16) with VUS and 46% (n=31) with BRCA mutations opted for CPM. **Conclusion:** Overall 32% of breast cancer patients with germline mutations or VUS opt for CPM at our institution. The rate for CPM in non- BRCA mutations carriers is high despite no available data regarding contralateral breast cancer risk and benefit of CPM. This finding should be validated in larger cohorts, including identification of reasons behind decision for CPM in these cohorts.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-10

Title: Breast health behaviors of women under 40 accessing the Avon breast health outreach program

Gates-Ferris K, Bauer KM M and Senter L. Cicatelli Associates Inc., New York, NY.

Body: Background

Breast cancer is the most common cancer among women, and tends to be more aggressive when diagnosed in women <40 years. However, limited research exists on breast health behaviors of women <40. This study analyzes trends in breast health behaviors and diagnostic data among women <40 served by the Avon Breast Health Outreach Program (BHOP) which links medically underserved women to breast health education and screening services.

Methods

Confidential client intake records of female clients (n= 192,296) of Avon BHOP grantees continuously-funded from 2011-2015 were analyzed, along with breast cancer diagnosis data from a select number of clients (n=82) from January-March 2016.

Results

Approximately 7% of Avon BHOP clients from 2011-2015 were <40 years (n=12,872). Women <40 had a significantly lower odds of having been taught breast self-exam (BSE), knowing about mammograms before their visit, having had a clinical breast exam (CBE) in the past 2 years, and having had a mammogram in the past 2 years compared to women ≥ 40 . Over 80% of women <40 reported being taught BSE and knowing about mammograms before their visit, compared to 94% among women ≥ 40 . The percent who reported a CBE in the past 2 years increased by approximately 5% among both <40 and ≥ 40 women, to a high of 64.6% and 78.2%, respectively, in 2015. Reporting a mammogram in the past 2 years also increased by approximately 5% in both groups, to a high of 25.9% and 70.3%, respectively, in 2015.

Women <40 had a significantly higher odds of having breast-related symptoms (OR=7.64) and a relative diagnosed with breast cancer (OR=1.23) compared to women ≥ 40 . For women <40, the adjusted odds of having had a mammogram in the past 2 years were 1.34 times higher ($p < 0.001$) among those who had a relative diagnosed with breast cancer compared to those who did not, while the odds were 1.09 ($p < 0.001$) among women ≥ 40 .

From January-March 2016, 10 breast cancer cases in women <40 were reported by 7 Avon BHOP grantees; 38 cases were reported in women ≥ 40 . Of the 10 diagnosed cases, the majority were Hispanic (70%) and 30% were Stage IV compared to 6% among women ≥ 40 , however the difference was not statistically significant ($p = 0.096$).

Discussion

In 2015 the American Cancer Society estimated that 4.2% of breast cancer cases were diagnosed in women <40. While the Avon BHOP diagnosis data is limited, the results nonetheless demonstrate a higher rate of breast cancer in this age group. Additionally, the association between being <40 and having a later stage diagnosis compared to those ≥ 40 appears to be a meaningful trend, and, while not significant in our preliminary data, we anticipate the finding to persist.

While Avon BHOP clients <40 had a significantly lower odds of knowing about screening behaviors compared to ≥ 40 , a large percentage had knowledge of breast health, and increasingly reported higher rates of screening behaviors. There was also a greater positive association between having a relative with breast cancer and having had a mammogram in the past 2 years in women <40 compared to ≥ 40 .

These findings suggest that Avon BHOP clients <40 increasingly understand their breast cancer risk and seek screening services accordingly. It is important to provide clear screening guidelines for this population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-11

Title: The association between angiotensin receptor blockers usage and breast cancer characteristics

Goldvaser H, Rizel S, Hendler D, Neiman V, Shepshelovich D, Shochat T, Sulkes A, Brenner B and Yerushalmi R. Rabin Medical Center, Petach Tokva, Israel.

Body: Background: Data regarding the impact of angiotensin receptor blockers (ARB) on breast cancer are inconsistent. We evaluate the association between ARB usage and breast cancer characteristics and outcomes.

Methods: All patients who were treated in our institute for estrogen receptor positive, human epidermal growth factor receptor 2 negative early breast cancer between 4/2005 and 3/2012 and whose tumors were sent for Oncotype-DX analysis were included. Medical records were retrospectively reviewed for clinical-pathological parameters, related comorbidities, treatment and outcomes. Data regarding ARB usage was retrieved. Usage of several pre-specified medications for hypertension including angiotensin converting enzyme inhibitors (ACEI), mineralocorticoid receptor antagonists (MRA), and β -blockers (BB) was also evaluated. Each medication group was compared to the rest of the study population.

Results: 671 patients were included in the study cohort. Forty six (7%) were treated with ARB, 93 (14.2%) with ACEI, 14 (2.1%) with MRA, and 115 (17.5%) with BB. ARB usage was associated with different histological subtype distribution ($P=0.009$), higher incidence of macroscopic nodal involvement ($P<0.001$) and more advanced stage at diagnosis ($p<0.001$). These findings remained significant on multivariate analysis. Patients treated with ARB had worse 5-year breast cancer specific survival (94.7% vs. 98.8%, $P=0.024$) and worse 5-year overall survival (94.6% vs. 98.8%, $p=0.015$), but these differences were not demonstrated on multivariate analysis ($p=0.251$ and $p=0.441$. respectively).

Conclusions: Patients treated with ARB presented with more advanced breast cancer disease and some distinct histological features. Further research is required to elucidate the effect of ARB treatment on breast cancer.

Tumor burden

Population (no.)	Tumor size, cm (SD)		Macroscopic node positive ¹		Stage		
	Mean, cm (SD)	P, univariate analysis	%	P, univariate analysis	I, %	II, %	P, univariate analysis
All (671)	1.68 (0.8)	-	9	-	71.2	28.3	-
ARB (46)	1.89 (0.81)	0.054	23.9	<0.001	37	63	<0.001
ACEI (93)	1.93 (0.93)	0.004	8.5	0.863	63.8	35.1	0.355
MRA (14)	1.88 (0.67)	0.329	14.3	0.363	57.1	42.9	0.907
BB (115)	1.77 (0.95)	0.216	13.2	0.087	66.7	31.6	0.091

¹Macroscopic nodes: lymph node metastases > 2 millimeter

Histological characteristics

Population (no.)	Ki67 (%)		Estrogen receptor stain intensity		Histology subtype		
	Mean, (SD)	P, univariate analysis	Mean, (SD)	P, univariate analysis	IDC (%)	ILC (%)	P, univariate analysis
All (671)	15.91 (13.58)	-	2.47 (0.57)	-	80.9	12.2	-
ARB (46)	12.27 (7.19)	0.005	2.57 (0.59)	0.246	65.2	26.1	0.009

ACEI (93)	17.1 (14.24)	0.403	2.44 (0.55)	0.515	75.5	16	0.358
MRA (14)	21.4 (11.2)	0.197	2.63 (0.42)	0.303	85.8	7.1	0.841
BB (115)	15.93 (12.93)	0.99	2.51 (0.58)	0.496	75.7	13.9	0.186

IDC- invasive ductal carcinoma, ILC- invasive lobular carcinoma

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-12

Title: The association between patient comorbidity and breast cancer survival

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Body: Background

There are studies suggesting significant association between the patient's comorbidity and perioperative outcomes. In breast cancer, patient comorbidity can also influence the oncologic outcome by affecting the decisions regarding adjuvant treatments. In this study, we examined the long-term oncologic outcome in breast cancer patients who underwent curative surgery according to their pre-existing comorbid conditions.

Methods

The medical records of 2,501 patients who underwent surgery for primary breast cancer from June 2006 to June 2010 were reviewed retrospectively. The patients were classified into three groups (ASA 1, 2, 3) according to preoperative ASA status determined by the anesthesiologists. Clinico-pathologic characteristics and survival outcomes of the patients were compared among the different co-morbidity groups.

Result

There were 1,792 (71.6%), 665 (26.6%), and 44 (1.8%) patients in ASA 1, 2, and 3, respectively. Total 95 (3.8%) deaths and 269 (10.8%) recurrences (loco-regional and distant) occurred during the median follow-up period of 71 months. Patients with high comorbidity showed significantly higher rate of death (51 (2.8%), 38 (5.7%) and 6 (13.6%) deaths in ASA 1, 2 and 3 group, respectively, $p < 0.001$). The ASA 3 patients also showed significantly higher rate of breast cancer recurrence when compared to other groups (180 (10.0%), 80 (12.0%) and 9 (20.5%) in ASA 1, 2 and 3, respectively, $p = 0.041$). Cox multivariate analysis demonstrated that high ASA score (ASA 3) was an independent prognostic factor in DFS, OS and BCSS (95% confidence interval [CI], 1.661-6.780; $p = 0.001$, 1.753-11.321; $p = 0.002$ and 2.222-18.429; $p = 0.001$). Fewer patients in the high co-morbidity group received adjuvant therapies (77 (4.3%), 44 (6.6%) and 8 (18.2%) in ASA 1, 2 and 3, $p < 0.001$). The increased recurrence of breast cancer in the high morbidity group was most seen in patients who did not receive adjuvant therapies. The incidence of serious adverse effect during the adjuvant therapy did not differ according to the co-morbidity conditions.

Conclusion

In this study, high comorbidity was related to increased risk of death and recurrence in breast cancer. The increased risk of recurrence in high co-morbidity group was mostly seen in patients who did not receive adjuvant therapies. In patients who underwent the adjuvant therapies, the incidence of serious adverse effects did not differ according to the co-morbidity status.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-13

Title: *TP53* gene polymorphisms (c.[215G>C]) in breast cancer patients and predisposition to family cancers- Single center experience

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Body: Introduction: Somatic mutations in the *TP53* gene are one of the most frequent alterations in human cancers, and germline mutations are the underlying cause of Li-Fraumeni syndrome, which predisposes to a wide spectrum of early-onset cancers. The aim of this study was to evaluate the association between *TP53* gene polymorphisms (c.[215G>C]) and predisposition to family cancers. **Methods:** We reviewed the medical records of 89 (21% *TP53* gene homozygotes and 79% heterozygotes) breast cancer patients who were diagnosed and treated in COI in Gliwice. The history of family cancers was collected from medical history. Mutation profile was assessed by RFLP-PCR technique. We evaluated the presence of polymorphism *TP53* (c.[215G>C]). Statistical analysis was carried out using STATISTICA 7 software. **Results:** Cancers in family history were detected in 72% of pts with *TP53* polymorphisms and they were observed insignificantly more often in heterozygotes than in homozygotes (76% vs. 58%, $p=0.153$). The most frequently reported cancers were: breast cancer (33%), lymphoma (17%), colorectal cancer (9%), OUN cancer (8%), gastric cancer (8%), pancreatic cancer (7%), ovarian cancer (6%) and renal cancer (3%). In analyzed group, renal cancers in family were detected more frequently in *TP53* homozygotes than in heterozygotes (16% vs. 0, $p=0.008$). Similarly, there was also observed tendency to the presence of colorectal cancer in *TP53* homozygotes (11% vs. 0, $p=0.193$). Ovarian cancers and cholangiocarcinomas in family were also observed insignificantly more often in homozygotes than in heterozygotes (7% vs. 0, $p=0.580$) and (5% vs. 1%, $p=0.383$). There was no association between number of cancers in family and *TP53* polymorphisms (47% vs. 43%, $p=0.797$). **Conclusion:** *TP53* polymorphism (c.[215G>C]) predisposed to development renal and colorectal cancers (*TP53* homozygotes) and ovarian cancer or cholangiocarcinoma (*TP53* heterozygotes) in family.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-14

Title: Geographic variation in the female breast cancer mortality rates across counties in United States

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Body: Background: Few studies have examined geographic disparities in breast cancer mortality at smaller geographical areas in the United States (U.S.) taking into account geographical details and spatial auto-correlations. Though these have identified counties with high breast cancer mortality risk, county-level socioeconomic attributes which are proven to be risk factors for breast cancer mortality were not examined and accounted for. The aim of this study is to efficiently map the spatial variation in the female breast cancer mortality rates across the counties of U.S., and to identify the high risk geographical clusters while adjusting for the county attributes, such as ethnic distribution of the population, educational attainment, poverty, unemployment and health care access.

Methodology: County-specific age standardized rates for breast cancer mortality for women aged ≥ 20 years in the U.S. were obtained for 3,109 counties from Surveillance Epidemiology and End Results (SEER) program from 1990-2012. We gathered county attributes from American Community Survey, such as the percentages of Hispanic white, Non-Hispanic white, Non-Hispanic black, < high school education, below 200% poverty, urban, foreign born, language isolation, women aged ≥ 40 years with mammography within last 2 years, and median household income. Then, we applied factor analysis to condense county attributes into three factors as covariates namely Hispanic immigrants, health care access among urban high income population, and non-Hispanic black unemployment. Spatiotemporal analysis was carried out by the structured additive regression model to incorporate spatial functions and Bayesian inference using Markov Chain Monte Carlo simulation techniques. Deviance Information Criteria was used for model comparisons and selections.

Results: Moran's index for the age standardized breast cancer mortality rate was 0.12 (p-value < 0.001) suggesting the existence of spatial dependence for the breast cancer mortality among the counties of U.S. Estimated effects [Mean (95%CI)] of spatial estimates for the factors were 0.03(0.02-0.05) for Hispanic immigrant culture, 0.06(0.04-0.09) for health care access among urban & high income groups and 0.41(0.31-0.51) for the non-Hispanic black unemployment. Counties in the Southwest region, Rocky mountain region and those in the western border of Midwest region of U.S with more Hispanic immigrants, have significantly lower mortality rates, a finding that can attributed to the lower incidence of breast cancer among Hispanics. As the mammography screening among urban and high income areas of the counties in the Mid-west region increase, the risk of breast cancer mortality increase significantly above the national average. And as the non-Hispanic black unemployment rates increase, the counties of Mid-West and those of South-West were at higher risk of mortality compared to national average. The percentage of counties with significant positive spatial function were 3.8% for the health care access factor and 6.3% for non-Hispanic black unemployment factor.

Conclusion: These initial results might explain social, cultural, and other reasons for the observed geographic variations, and in turn, could support a stronger theoretical basis for public health policy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-15

Title: Economic impact of breast cancer in Mexico

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Body: Background: Breast cancer is the most prevalent cancer in Mexico and is the leading oncologic death cause among women older than 25 years-old. Its incidence has steadily increased during the last decades. Unfortunately, most of the patients are diagnosed in advanced stages, increasing the cost of the treatment and mortality. Through this study, we analyse the annual economic impact of breast cancer in Mexico.

Materials and methods: Data from Mexican official statistics from 2014 were obtained. The rate of population covered by the public health system, the mean income *per capita*, the age for retirement, the funds used in public hospitals according to clinical stages, the mean age for diagnosis and the mean age of death by breast cancer in Mexico and the mean cost of a funeral service were obtained.

We calculate the direct costs including the funds used for diagnosis and treatment of the patients with breast cancer in the public hospitals. The indirect costs were obtained calculating the mean *per capita* income lost due to death by breast cancer, the number of deaths before 65 years-old (Age for retirement in Mexico) and the mean cost for funeral services.

Costs for breast cancer treatment were also obtained according to the funds for early stages (0 – IIA), locally-advanced stages (IIB – IIIC) and metastatic stages (IV). Results were compared between groups.

Results: In 2014, around 20,500 new cases of breast cancer were diagnosed in Mexico. Among them, 15,826 patients (77.2%) were treated in public health institutions. From these patients, 38.7% were diagnosed in early stages, 51% in locally-advanced stages and 9.6% in stage IV. Only 22% were diagnosed during screening mammography.

In 2014, funds for early stages amounted to US \$44,077, 959; for locally-advanced stages to US \$120,727,505 and for metastatic stages to US \$ 192,623,862. The total fund for breast cancer, including diagnosis and treatment, was US \$ 304, 747,165.

Treatments for locally advanced stages were 107.8% higher compared to early stages (US \$14,958 / 7,197).

It has been demonstrated that the mean age of breast cancer diagnosis in Mexico is 53 years-old, while the mean age of death due to this disease is 58 years-old. Age for retirement is 65 years and 76% of the breast cancer patients appeared in this group of age or younger. The mean per capita income is US \$22 and the cost for funeral was US \$1,968 approximately. The mean Overall-Survival rate for breast cancer in Mexico is 83.1%, so the mortality rate is 16.9%, so 2044 patients died due to breast cancer in 2014. The economic loss for the families amounted to US \$120,647,834: US\$116, 624,822 for the loss of the *per capita income* and US \$4,023,012 for the cost for funeral services.

Conclusions: Breast cancer has a great economic impact in both the government and the patient, especially in advanced stages. It is extremely important to improve the mammographic screening programs in Mexico and reduce the time of care. Initiation of annual screening mammography starting at 40 years-old should be a mandatory policy in order to increase the rate of diagnosis of breast cancer at early stages. In the near future, there will not be sufficient economic resources for treating breast cancer in Mexico, if new and strict policies are not carried out.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-07-16

Title: Alcohol consumption increases mammographic density in women aged ≥ 55 years

Uchida K, Ohashi H, Kitano N, Tsunoda K, Nagamatsu T, Sekine C, Imawari Y, Nogi H and Takeyama H. Meiji Yasuda Life Foundation of Health and Welfare, Shinjuku-ku, Tokyo, Japan and The Jikei University School of Medicine, Minato-ku, Tokyo, Japan.

Body: Background: Increased mammographic density is a significant risk factor for breast cancer and decreases the sensitivity of mammography screening. The aim of this study is to examine the factors affecting breast density in Japanese women.

Data sources and methods: Between Apr. 2014 and Mar. 2016, 3492 women were received mammography screening. According to the results of mammography, breast density was categorized as non-dense(BI-RADS 1 and 2, n=1670) and dense(BI-RADS 3 and 4, n=2222) . Logistic regression was used to estimate the odds of having dense breasts by age, body mass index(BMI), alcohol consumption, smoking, parity, menopausal status, dysmenorrhea, hormone use, family history of breast cancer, physical activity, fried foods intake, brightly colored vegetables intake, coffee intake and tea or green tea intake. All statistical tests were two-sided.

Results: There was a statistically significant negative interaction of age(OR=0.97, <0.001), BMI(OR=0.78, <0.001), number of live birth (one; OR=0.77, <0.030, two or more; OR0.37, <0.001), post-menopause(OR=0.6, <0.001) on breast density

Results of analysis of factors influencing breast density among all study participants

		Odds ratio	95% CI	P
Age	Continuous variables	0.97	0.95-0.98	<0.001
BMI	Continuous variables	0.78	0.76-0.80	<0.001
Alcohol intake	none	1.00		0.325
	<20g a day	1.12	0.94-1.35	0.209
	≥ 20 g a day	1.21	0.91-1.60	0.186
Smoking	no	1.00		
	yes	0.94	0.71-1.23	0.644
Birth	0	1.00		<0.001
	once	0.77	0.61-0.97	0.030
	≥ 2	0.37	0.31-0.45	<0.001
Menopause	pre	1.00		
	post	0.60	0.47-0.77	<0.001
Dysminorrhea	no	1.00		
	yes	0.78	0.41-1.48	0.446
Family history of breast cancer	no	1.00		
	yes	1.00	0.81-1.24	0.991
Physical activity	no	1.00		
	yes	0.89	0.74-1.07	0.207
Fried foods intake	none	1.00		0.087
	once two days	0.78	0.60-1.02	0.068
	\geq once a day	0.76	0.51-1.12	0.166
Brightly coloredd vegetables	none	1.00		0.910
	once two days	0.95	0.70-1.28	0.737
	\geq once a day	0.95	0.73-1.22	0.666

Coffee intake	none	1.00		0.105
	once two days	1.06	0.75-1.51	0.729
	≥once a day	1.22	1.01-1.47	0.040
Tea/Green tea intake	none	1.00	1.01-1.47	0.098
	once two days	0.76	0.55-1.05	0.097
	≥once a day	0.84	0.70-1.00	0.055
bone mineral density(n=510)	<80%	1.00		
	≥80%	0.63	0.38-1.07	0.068

. On the other, there was a statistically significant positive interaction of alcohol intake(20g or more a day, OR=1.62, 0.026) in dense breast women aged ≥55 years

Results of analysis of factors influencing breast density in women aged ≥55 years (n=1382)

		Odds ratio	95% CI	P
BMI	<25kg/m ²	1.00		
	≥25kg/m ²	0.30	0.21-0.44	<0.001
Birth	0			Trend P<0.001
	once	0.69	0.46-1.04	0.0076
	≥two	0.31	0.23-0.41	<0.001
Alcohol intake	none			0.052
	<20g/day	1.26	0.98-1.62	0.074
	≥20g/day	1.62	1.06-2.48	0.026

. A trend of bone mineral density ≥80% and dense breast was seen in women aged ≥40 years(OR=0.63, <0.086, n=510).

Conclusion: Alcohol consumption increase mammographic breast density in women aged ≥55 years. As both of increased breast density and alcohol consumption have been suggested to increase risk of breast cancer, more cautious mammographic screening should be considered for those women aged ≥55 years.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-08-01

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-08-02

Title: Progression-free survival results in patient subgroups from a Phase 3 randomized trial of fulvestrant 500 mg vs anastrozole for hormone receptor-positive advanced breast cancer (FALCON)

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Body: BACKGROUND

Improved efficacy was suggested for fulvestrant 500 mg, a selective estrogen receptor degrader (SERD), vs anastrozole as first-line treatment for hormone receptor-positive locally advanced or metastatic breast cancer (LA/MBC) in the open-label Phase 2 FIRST trial. The Phase 3, randomized, double-blind, multicenter FALCON trial (NCT01602380) compared fulvestrant 500 mg with anastrozole 1 mg in patients with hormone receptor-positive LA/MBC who had not received prior hormonal therapy. The primary endpoint of the study was met, such that there was a statistically significant improvement in progression-free survival (PFS) for fulvestrant 500 mg vs anastrozole. We present an analysis of PFS for pre-specified patient subgroups in the FALCON trial.

METHODS

Eligible patients had ER and/or progesterone receptor (PgR)-positive breast cancer, WHO performance status 0–2 and ≥ 1 measurable/non-measurable lesion(s). Patients were randomized (1:1) to receive fulvestrant 500 mg (IM on Days 0, 14, 28, and each 28 days thereafter) or anastrozole 1 mg daily. The primary endpoint was PFS, assessed via RECIST 1.1, surgery/radiotherapy for disease worsening, or death (any cause). PFS was evaluated in patient subgroups defined by pre-specified baseline covariates. The consistency of effect across patient subgroups was assessed via hazard ratios and 95% confidence intervals using a log-rank test.

RESULTS

Overall, 462 patients were randomized to treatment: 230 received fulvestrant 500 mg and 232 received anastrozole. PFS outcomes in each patient subgroup are presented in the Table.

CONCLUSIONS

This analysis of patient subgroups from the FALCON trial suggests that treatment effects were largely consistent across the subgroups analyzed with some possible exceptions (e.g. patients with visceral vs non-visceral disease). Further work is ongoing to understand the possible treatment effect in these subgroups.

PFS in pre-specified patient subgroups

Subgroup	Number of patients (%) with event		Hazard ratio (95% CI)
	Fulvestrant 500 mg n=230	Anastrozole n=232	
Breast cancer type			
Locally advanced	11/28 (39.3%)	14/32 (43.8%)	0.79 (0.36, 1.73)
Metastatic	132/202 (65.3%)	152/200 (76.0%)	0.78 (0.62, 0.99)
Prior chemotherapy for LA/MBC			
Yes	31/36 (86.1%)	33/43 (76.7%)	1.08 (0.66, 1.77)
No	112/194 (57.7%)	133/189 (70.4%)	0.75 (0.59, 0.97)
Geographic region^a			
US/Canada	16/25 (64.0%)	19/24 (79.2%)	0.66 (0.34, 1.30)
Non-US/Canada	127/205 (62.0%)	147/208 (70.7%)	0.81 (0.64, 1.03)

Measurable disease			
Yes	124/193 (64.2%)	143/196 (73.0%)	0.76 (0.60, 0.97)
No	19/37 (51.4%)	23/36 (63.9%)	0.99 (0.53, 1.82)
ER-positive and PgR-positive			
Yes	103/175 (58.9%)	127/179 (70.9%)	0.73 (0.56, 0.94)
No	40/55 (72.7%)	39/53 (73.6%)	1.04 (0.67, 1.62)
Bisphosphonate use at baseline			
Yes	44/61 (72.1%)	53/62 (85.5%)	0.69 (0.46, 1.03)
No	99/169 (58.6%)	113/170 (66.5%)	0.82 (0.63, 1.07)
Visceral disease			
Yes	92/135 (68.1%)	87/119 (73.1%)	0.99 (0.74, 1.33)
No	51/95 (53.7%)	79/113 (69.9%)	0.59 (0.42, 0.84)

^aData for Asia/non-Asia subgroups are presented in a separate abstract

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-08-03

Title: Targeting inflammatory pathways: A phase 2 trial of the JAK-inhibitor ruxolitinib in combination with exemestane for aromatase inhibitor-resistant, estrogen receptor-positive breast cancer

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Body: Background: *In vitro* mechanisms link IL-6 to poor outcome in breast cancer via inflammatory pathways, activated JAK/STAT tumor signaling and upregulation of aromatase, leading to an aggressive tumor phenotype. Epidemiological data from our group and others support these mechanisms in women with ER-positive (+) disease. We therefore hypothesized that the JAK inhibitor, ruxolitinib (RUX, INCB018424; Incyte), would enhance activity of exemestane (EXE) in women with ER+ breast cancer who relapsed after non-steroidal aromatase inhibitor therapy, particularly among carriers of a germ-line polymorphism in IL-6, conferring elevated levels of IL-6 in the tumor microenvironment.

Methods: The “JAKEE trial” is a phase II trial to determine the safety and efficacy of RUX + EXE in postmenopausal women with relapsed, ER+ advanced breast cancer. Eligible patients were required to have progressed on a non-steroidal AI and either measurable or bone-only disease. CRP, a putative biomarker of tumor microenvironment inflammation, was measured at baseline and serially during treatment. Using a Simon 2-stage design, we treated 15 patients with RUX at 25 mg BID and EXE at 25mg daily on a continuous 28-day schedule. First stage results were previously presented (AACR, 2014). Accrual proceeded to second stage after no patient met the pre-defined stopping rule of grade (G) 3/4 toxicity requiring discontinuation from the study within the first treatment cycle. Due to the substantial rate of anemia requiring dose reductions, however, RUX dose was reduced to 15 mg BID in second stage.

Results: A total of 25 patients were enrolled; 24/25 had progressed on AI in metastatic setting; 1 relapsed on adjuvant AI. RUX+EXE was well-tolerated overall, with only 2 G4 events (creatinine elevation, hepatic failure); both were due to disease progression. 16% had G3 fatigue, anemia or hypertension; 12% had G3 neutropenia or depression. Other lower grade toxicities in >20% included musculoskeletal pain, increased ALT, and headache. Overall, patients stayed on therapy for a median of 3 cycles (range 2 – 21). There were no CR or PR, but 6/25 (24%) had prolonged disease control ($SD \geq 6$ months). Median CRP at study entry was 6.4 (range 0.3-38.9), with 8/25 (32%) having $CRP \geq 10$. Achieving $SD \geq 6$ months was not associated with baseline CRP ($CRP \geq 10$ in 32% with vs. 33% without $SD \geq 6$ months, $p(\text{exact})=1.0$). A novel pharmacodynamic assay to assess STAT3 phosphorylation in peripheral blood mononuclear cells after RUX exposure demonstrated differential effects in patients with response.

Conclusions: Targeting JAK/STAT signaling in AI-resistant breast cancer with RUX+EXE was safe and well-tolerated. 24% of patients had prolonged SD, but baseline CRP level did not predict response. Correlative studies to determine whether host and/or tumor biomarkers predict response to therapy, including germline IL-6 genotype, immune profiles, p-STAT3 and estradiol levels, are currently underway.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-08-04

Title: Phase 1/2 study of oral seviteronel (VT-464), a dual CYP17-lyase inhibitor and androgen receptor (AR) antagonist, in patients with advanced AR positive triple negative (TNBC) or estrogen receptor (ER) positive breast cancer (BC)

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Body: Background:

Seviteronel (Sevi), a CYP17-lyase (L) inhibitor (reduces testosterone (T) and estradiol (E2) biosynthesis) and a competitive AR antagonist, has activity in castration resistant prostate cancer at a dose of 600mg nightly. Sevi potently inhibits the growth of ER(+)/AR(+) MCF7, tamoxifen-resistant (TAMR) MCF7, and ER(-)/AR(+) MDA-MB-453 cells. In a TAMR xenograft BC model, Sevi decreases tumor growth greater than enzalutamide (Enza), an AR antagonist (Ellison et al, SABCS 2015). Nearly all subtypes of BC, including AR(+) TNBC, are potential targets for Sevi based on its mechanism of action (MOA). Phase (Ph) 1 of this study established the recommended Ph 2 dose (RP2D) of Sevi in women with BC as 450mg once nightly, based upon preliminary tolerability and pharmacokinetics (PK) (Bardia et al, ASCO 2016). The primary objective of Ph 2 is to estimate the activity of Sevi, as measured by clinical benefit rate (CBR) at 16 and 24 weeks (wks) for AR(+) TNBC and ER(+) BC, respectively. The secondary objectives include an estimation of Sevi tolerability and pharmacodynamics (PD) (NCT02580448).

Methods:

Women with advanced AR(+) TNBC (stratified by prior Enza use) or ER(+) BC were enrolled using 3 parallel Simon's 2-stage designs powered to evaluate CBR. ER(+) BC patients must have had ≥ 1 prior line of endocrine therapy; no limit to prior treatment for TNBC. AR(+) status was confirmed using central IHC analysis in all patients, with a $\geq 10\%$ tumor cell nuclear staining cutoff for evaluable TNBC patients. Sevi was administered once nightly with dinner at 450mg (28d cycle). Tumor and blood samples were collected for PK and PD analysis (circulating tumor cells, ctDNA, sex steroids). Response was assessed every 8 wks for 52 wks, then every 12 wks thereafter. Current tolerability and PD results are presented herein for this ongoing Ph 2 study.

Results:

As of June 7, 2016, 17 patients received Sevi at 450mg nightly between Ph1 and Ph2 with 10 in screening. 14 patients are currently on study in Cycles 1-6. The most common adverse events (AEs > 10% regardless of causality or grade) were tremor (24%), pain (18%), fatigue (18%) and dyspnea (18%), nausea (12%), AST increase (12%), ALT increase (12%) and abdominal pain (12%), all of which were Grade 1 or 2 except for Grade 3 dyspnea (n=1; unrelated). No dose reductions were reported and there were no drug-related discontinuations. Nine patients underwent central AR testing (4 AR(+) of 6 TNBC; 3 AR(+) of 3 ER(+) BC). Median AR tumor cell nuclear staining was 90% (15-100%). Preliminary sex steroid analyses from 6 Ph 1 patients receiving Sevi at 450, 600, or 750mg nightly (n=2 at each dose) for 1 cycle showed a median decline in E2 concentration of 52% (-29 to -87%) to 12.4pmol/L (4 to 33pmol/L) from baseline. There was a similar magnitude of decline for T.

Conclusions:

Sevi was well-tolerated at 450mg nightly with exposures similar to the RP2D in men (600mg nightly). The CYP17-L inhibition activity of Sevi was demonstrated with an early and potent reduction in E2 and T. Sevi's unique CYP17-L and AR antagonist MOA may provide a new novel treatment option for AR(+) TNBC or ER(+) BC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-08-05

Title: Phase I/II trial of palbociclib in combination with bicalutamide for the treatment of androgen receptor (AR)+ metastatic breast cancer (MBC)

Gucalp A, Corben AD D, Patil S, Feigin KN N, Boyle LA, Hudis CA A and Traina TA A. Memorial Sloan Kettering Cancer Center, New York, NY.

Body: Background: Triple negative breast cancer (TNBC) represents 15% of all BCs. Conventional chemotherapy remains the standard, albeit inadequate, option for these patients (pts) as evidenced by poor disease free and overall survival. The androgen-signaling pathway plays a role in BC pathogenesis and emerging evidence suggests the androgen receptor (AR) is a therapeutic target. TBCRC011 established safety and efficacy of inhibiting AR with bicalutamide (B) in patients with AR+/ER/PgR- metastatic BC (MBC). The regimen was well-tolerated and the study met its prespecified efficacy endpoint demonstrating a clinical benefit rate (CBR) of 19% in this population (Gucalp et al. CCR 2013). The rationale for combining palbociclib (P) with AR blockade stems from prior work in ER+ MBC. P is an oral, selective inhibitor of CDK4/6 activity which significantly improved median progression-free survival (PFS) in combination with letrozole compared to letrozole monotherapy for the treatment of postmenopausal pts with ER+ MBC in the first-line setting. Consistent with preclinical data, P has been shown to reduce growth of AR+ ER/PgR- MDA-MB-453 breast cancer cells via reduced Rb phosphorylation. It has been shown that AR+ TNBC expresses a luminal profile and has intact Rb protein, the target of palbociclib activity. Therefore, we hypothesize that P will increase the efficacy of B in pts with metastatic AR+ TNBC. NCT02605486.

Methods: Pts with AR+ (IHC \geq 1%)/ER any/HER2(-) MBC on central review at MSK were eligible if met following criteria: ECOG \leq 2, postmenopausal, no limit to prior regimens. Pts with ER+ BC must have had 1 prior endocrine therapy. Treatment: B orally daily and P orally daily 3 weeks on 1 week off. DLT period = 28 days. Pts are evaluated for toxicity every 2-4 weeks and for response every 8 weeks. Ph I standard 3+3 design with 3 dose escalations. The primary objective of the phase I portion of the study is to determine the recommended phase II dose of P in combination with B. Plasma for PK was collected throughout the study.

Results: As of 6/12/16, 9 pts with AR+ MBC are enrolled. Accrual is complete to the second dose escalation cohort and the final dosing cohort of B 150mg + P 125 mg began enrollment as of 5/24/16. PK analysis is ongoing and data are expected for presentation. Treatment has been well tolerated with no related Grade 4 or 5 AEs. One SAE of Grade 3 anemia (thought to be related to P) and Grade 4 hypercalcemia led to hospitalization deemed related to disease progression.

Conclusions: The combination of B+P has been well tolerated with no unexpected toxicity observed. Updated safety, response, and PK data will be presented.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-08-06

Title: A phase 1 study of RAD1901, a novel, oral selective estrogen receptor degrader, for the treatment of ER-positive advanced breast cancer

Kaklamani VG G, Kabos P, Elledge R, Harb W, Purandare D, O'Neill A, Garner F and Bardia A. University of Texas Health Science Center at San Antonio, San Antonio, TX; University of Colorado Denver, Aurora, CO; Horizon Oncology Center, Lafayette, IN; Radius Health, Inc., Waltham, MA and Massachusetts General Hospital, Boston, MA.

Body: Background: The majority of breast cancers are defined as estrogen receptor positive (ER+) breast cancer. Despite availability of standard therapies, such as aromatase inhibitors, many women eventually relapse with aggressive disease due to acquisition of endocrine resistance, including ESR1 mutations. To help address some of the challenges associated with current therapies including exposure limitations and intramuscular administration, we have developed RAD1901, a novel, non-steroidal, oral selective estrogen receptor degrader (SERD). Preclinical studies with RAD1901 have demonstrated a dose dependent degradation of ER consistent with a SERD mechanism of action. In multiple in vivo models of breast cancer, including patient-derived xenograft models that are sensitive or resistant to standard endocrine therapies, RAD1901 has anti-tumor efficacy both as a single agent and in combination with palbociclib and everolimus. Importantly, RAD1901 has shown superior efficacy compared to fulvestrant in a number these models including those harboring and ESR1 mutation.

Methods: RAD1901-005 is a Phase 1 study currently enrolling ER+ advanced metastatic breast cancer patients (ClinicalTrials.gov identifier: NCT02338349) with a dose escalation cohort based on a standard 3+3 design followed by a safety expansion cohort at a tolerated dose. Key inclusion criteria include postmenopausal women aged 18 years or older, with advanced ER+, HER2-negative breast cancer, who have received ≤ 2 prior chemotherapy regimens in the metastatic setting and > 6 months of prior endocrine therapy. In addition, circulating tumor DNA (ctDNA) was evaluated to determine ESR1 mutation status and to correlate it with clinical response.

Results: As of the cut-off date in March, 13 patients were enrolled in the dose escalation part of the study (3+3 design) at doses of 200 mg qd, 400 mg qd and 600 mg qd. RAD1901 exposure was dose dependent and the PK profile was comparable to PK data from a previous study in healthy volunteers. RAD1901 was well tolerated with the most common adverse events being low-grade nausea and dyspepsia. No DLTs were observed. A safety expansion cohort (Part B, n=20) was opened. At the cut-off date 4 of the 13 patients had been on study 4 or more months. Updated outcomes and biomarker data, including ctDNA, will be presented at the meeting.

Conclusion: RAD1901, a novel, non-steroidal, oral SERD, is well-tolerated with manageable adverse effects, and is associated with preliminary evidence of clinical activity in patients with advanced ER+ advanced postmenopausal breast cancer, including patients with ESR1 mutant tumors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-08-07

Title: Anti-proliferative and anti-inflammatory estrogen receptor agents for treatment of endocrine-resistant breast cancer

Josan JS S, Pokludova K, Devi S, Srinivasan S, Katzenellenbogen JA A and Nettles KW W. Virginia Tech, Blacksburg, VA; University of Illinois at Urbana-Champaign, Urbana, IL and The Scripps Research Institute, Jupiter, FL.

Body: Breast cancer is a challenging disease, because resistance invariably develops to front line therapies targeting estrogen receptor (ER, 70% of breast cancers are ER+), and some endocrine therapies have serious side effects, such as bone loss with aromatase inhibitors. Activated ER inhibits inflammatory gene expression *via* protein-protein interactions that block NF- κ B activity in ER+ tissues. Importantly, NF- κ B is a primary mediator of resistance in many cancers, including breast cancer. However, all current endocrine treatments (tamoxifen, raloxifene, fulvestrant) block this palliative signaling pathway, along with blocking the desired proliferative pathway. *Thus, there is a significant unmet clinical need for novel endocrine treatments for breast cancer that can ameliorate patient outcome in resistant population, be less prone to resistance development, retain the palliative effects that estrogens have on the inflammatory axis, and cause fewer side effects.*

We hypothesized that NF- κ B selective ER α antagonists will prove effective in treating endocrine-resistant breast cancer *in vivo*. Through ligand class analysis, we identified a **unique binding hotspot in ER α** that has allowed us to dial out the gene activation/proliferative signal of ER ligands, while retaining the strong anti-inflammatory properties of estradiol (E2). Using a novel screening approach based upon high-throughput x-ray crystallography, we previously identified a unique ligand scaffold, termed **OBHS** (for 7-oxa-bicyclo[2.2.1]heptene sulfonate for the prototypical member), which directs a side chain in this hotspot and reduces gene activation through ligand-induced shifts in helix 11 of ER. While SERMs directly reposition helix 12, OBHS compounds indirectly modulate helix 12 by regulating its docking onto helix 11, leading us to call them as *indirect antagonists*. Compounds in this novel class act in a mechanistically and structurally distinct fashion from the current endocrine therapies. One of the OBHS analogs surpasses the lead as well as estradiol (E2) for its anti-inflammatory activity mediated through ER. We further established GREB1 transcriptional model in our portfolio to predict response to endocrine therapy. GREB1 is likely a key regulator of estrogen-induced breast cancer growth, and has potential as a new biomarker for predicting E2-dependent and anti-E2-responsive breast cancers. OBHS analogs inhibit GREB1 transcription better than the OBHS parent lead. We are presently carrying out selective estrogen-receptor modulator (SERM)-like modifications to further improve anti-proliferative activity. To aid optimization, a computational workflow, based upon induced fit docking, has been developed. ***The ultimate impact of this research will be the development of novel anti-proliferative/anti-inflammatory ligands with unique and persistent efficacy as therapeutics for endocrine-resistant breast cancer, both in adjuvant and advanced disease settings.***

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-08-08

Title: A phase 1 study of RAD1901, an oral selective estrogen receptor degrader, to determine changes in the ¹⁸F-FES uptake and tumor responses in ER-positive, HER2-negative, advanced breast cancer patients

de Vries EGE GE, Venema CM M, Glaudemans AWJM WJM, Jager A, Garner F, O'Neill A, Patki A and Menke-van der Houven van Oordt CW. University Medical Center Groningen, Groningen, Netherlands; Erasmus Medical Center, Rotterdam, Netherlands; Radius Health, Inc., Waltham, MA and Free University Medical Center, Amsterdam, Netherlands.

Body: Approximately 75% of the tumors from breast cancer patients express the estrogen receptor (ER) at diagnosis. Since patients with ER-positive tumors respond to endocrine therapy, determination of the ER status of a patient has important consequences for treatment decision making. The current standard practice to assess ER status is by using immunohistochemical staining of a tumor biopsy. However, the ability to collect serial tumor biopsies during treatment to determine a patient's response to endocrine therapy is limited by the feasibility of performing multiple biopsies. ¹⁸F-fluoroestradiol positron emission tomography (FES-PET) is an imaging modality which can measure ER levels based on FES uptake and has been previously utilized to assess patients' response to endocrine therapies. Serial imaging of tumor FES uptake during treatment with the selective ER degrader (SERD) fulvestrant, demonstrated that significant residual FES uptake in tumor lesions was associated with early disease progression in patients with metastatic breast cancer (van Kruchten et al, Cancer Discov 2015;5:72-81).

RAD1901 is a novel, orally available SERD that binds to both ER mutant and wild type forms leading to ER degradation in preclinical models of breast cancer. A prior clinical study of RAD1901 in healthy postmenopausal women demonstrated decreased FES uptake in the uterus after RAD1901 treatment, further supporting the hypothesis that FES-PET imaging can provide an early indication of target effect with this agent.

RAD1901-106 is a Phase 1 study of RAD1901 in metastatic breast cancer patients to explore the use of FES-PET imaging as an early indicator of clinical response to RAD1901 treatment. Key inclusion criteria include postmenopausal patients with advanced ER-positive, HER2-negative breast cancer, who have received ≤ 3 prior line of endocrine therapy in the metastatic setting. Serial FES-PET imaging performed pre-treatment and after 14 days on therapy is used to evaluate the effect of RAD1901 treatment on the availability of ER binding sites in tumor lesions. Patients may continue on RAD1901 therapy until tumor progression. Patient enrollment started in early 2016 and is ongoing. FES-PET tumor imaging and preliminary clinical data from patients enrolled in the study will be presented.

ClinicalTrials.gov identifier: NCT02650817.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-08-09

Title: Progression-free survival results in postmenopausal Asian women: Subgroup analysis from a phase 3 randomized trial of fulvestrant 500 mg vs anastrozole for hormone receptor-positive advanced breast cancer (FALCON)

Shao Z, Ellis MJ J, Robertson JFR FR, Grinsted LM M, Fazal M and Noguchi S. Fudan University Shanghai Cancer Center, Shanghai, China; Lester and Sue Smith Breast Center, Baylor Clinic, Baylor College of Medicine, Houston, TX; University of Nottingham, Royal Derby Hospital Centre, Derby, United Kingdom; AstraZeneca, Cambridge, United Kingdom; AstraZeneca, Gaithersburg, MD and Osaka University Graduate School of Medicine, Osaka, Japan.

Body: BACKGROUND

Fulvestrant is a selective estrogen receptor degrader (SERD) with no known agonist effects. In the open-label Phase 2 FIRST study, fulvestrant 500 mg suggested improved efficacy as first-line treatment vs anastrozole in patients with hormone receptor-positive locally advanced or metastatic breast cancer (LA/MBC). The Phase 3, randomized, double-blind, multicenter FALCON trial (NCT01602380) compared fulvestrant 500 mg with anastrozole 1 mg in patients with hormone receptor-positive LA/MBC who had not received prior hormonal therapy. The primary endpoint of the study, progression-free survival (PFS) assessed via RECIST 1.1, surgery/radiotherapy for disease worsening, or death (any cause), was met, as shown by a statistically significant improvement in PFS for fulvestrant 500 mg vs anastrozole. This analysis evaluated PFS in the Asian patient subgroup, which included all randomized patients from centers in China, Japan, and Taiwan.

METHODS

Eligible patients had ER and/or progesterone receptor-positive breast cancer, WHO performance status 0–2, and ≥ 1 measurable/non-measurable lesion(s). Patients were randomized (1:1) to receive fulvestrant 500 mg (IM on Days 0, 14, 28, and each 28 days thereafter) or anastrozole 1 mg daily, and were stratified according to LA or MBC; prior or no prior treatment with chemotherapy for LA/MBC; and measurable or non-measurable disease. The consistency of effect across patient subgroups was assessed via hazard ratios and 95% confidence intervals using a log-rank test.

RESULTS

In total, 462 patients were randomized (n=230 fulvestrant 500 mg; n=232 anastrozole). The Asian subgroup comprised 67 patients (n=34 fulvestrant 500 mg; n=33 anastrozole). PFS outcomes for the Asian and non-Asian subgroups are presented (Table). The most commonly reported adverse event (AE) was arthralgia (18.2% vs 12.1% of patients with fulvestrant 500 mg and anastrozole, respectively). The rate of AEs leading to discontinuation of treatment was 3.0% and 3.0%, respectively.

CONCLUSIONS

Based on a preliminary assessment of 67 patients, the treatment effect in the Asian patient subgroup from the FALCON trial appears to be broadly consistent with the non-Asian population.

PFS in Asian and non-Asian patient subgroups

Geographic region	Number of patients (%) with event		Hazard ratio (95% CI)
	Fulvestrant 500 mg n=230	Anastrozole n=232	
Asia	19/34 (55.9%)	22/33 (66.7%)	0.81 (0.44, 1.50)
Non-Asia	124/196 (63.3%)	144/199 (72.4%)	0.79 (0.62, 1.01)

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-08-10

Title: Time-evolution of the efficacy of first line aromatase inhibitors (AI) in hormone receptor positive (HR+) advanced breast cancer (ABC): A moving target

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Body: INTRODUCTION. Recently presented studies suggest significant improvements in efficacy of endocrine therapy trials addressing HR+ ABC. Overall, progression-free survival (PFS) has consistently improved when we analyze first line endocrine trials. This study aims to evaluate the evolution of the PFS obtained with first line AI monotherapy in patients with HR+ ABC.

METHODS. We performed a systematic review of the literature searching for randomized clinical trials of endocrine therapy in HR+ ABC. All trials published since 2000 that involved a first line AI single agent arm were included. Median PFS, year of publication, sample size and population characteristics such as previous endocrine therapy exposure and presence of visceral metastases were extracted from each study and linear correlation used to access the trend of PFS along time.

RESULTS. 13 studies were identified accounting for 3,446 postmenopausal patients that received monotherapy with an AI as first line treatment for HR+ ABC. The year of publication/presentation ranged from 2000 to 2016. A total of 14 treatment arms exploring AI monotherapy were identified and analyzed. One study compared exemestane to anastrozole. The other 12 studies compared AIs as single agents to other endocrine strategies. Single agent anastrozole was used in six studies while letrozole was used in five and exemestane in one trial. PFS ranged from 8.2 months in the experimental arm treated with anastrozole in a trial published in 2000 to 15.6 in the control group treated with letrozole monotherapy in a study published in 2015. Linear regression showed a significant improvement in PFS over time ($p = 0.034$).

Table 1: studies characteristics

FIRST AUTHOR	AI	YEAR OF PUBLICATION	PHASE	N	PFS (m)	HT NAIVE POP (%)	VISCERAL METS (%)
BONETTERE	ANASTROZOLE	2000	3	340	8.2	88	33
NABHOLTZ	ANASTROZOLE	2000	3	171	11.1	80	47
MOURIDSEN	LETROZOLE	2001	3	453	9.4	81	45
PARIDAENS	EXEMESTANE	2008	3	182	9.9	90	47
ROBERTSON	ANASTROZOLE	2010	2	103	13.1	75	51
MEHTA	ANASTROZOLE	2012	3	345	13.5	60	49
BERGH	ANASTROZOLE	2012	3	256	10.2	33	51
MARTIN	LETROZOLE	2012	3	166	14.4	48	47
JOHNSTON	ANASTROZOLE	2013	2	104	14.0	NR	NR
WOLFF	LETROZOLE	2013	3	555	9.0	60	NR
IWATA	EXEMESTANE	2013	3	149	13.8	NR	50
IWATA	ANASTROZOLE	2013	3	149	11.1	NR	50
FINN	LETROZOLE	2014	2	81	10.2	72	48
DICKLER	LETROZOLE	2015	3	170	15.6	52	74
FINN	LETROZOLE	2016	3	222	14.5	43	50

CONCLUSION. A combination of improved medical care and patient selection factors probably explain most of this progressive

improvement in PFS obtained with the same therapeutic strategy. While in general, entry criteria have not changed significantly over time, more recent clinical trials addressing first line treatment of HR+ ABC are probably including a different population of patients. Exclusion of HER2 positive tumors and of unknown hormone receptor status patients as well as the percentage of patients without previous adjuvant exposure to endocrine therapy, for example, should be taken into account as they select for a more endocrine sensitive group of patients. This observation has implications in the planning of future studies, the estimation of sample size based in expected efficacy as well as the interpretation and generalization of the results to clinical practice.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-08-11

Title: An orally bioavailable selective estrogen receptor downregulator

Wang G, Liu J, Zheng S, Miele L, Wiese T, Zhong Q and Guo S. Xavier University of Louisiana, New Orleans, LA and LSU Health Sciences Center, New Orleans, LA.

Body: Orally bioavailable selective estrogen receptor downregulators (SERDs) may offer greater systemic drug exposure, improved clinical efficacy, and more durable treatment outcome for breast cancer patients with disease progression following antiestrogen or aromatase inhibitor therapy. We report the design and synthesis of ZB716, a C-3 position boronic acid modified fulvestrant, which behaves as a steroidal SERD suitable for oral administration. ZB716 binds to ER α competitively at an IC₅₀ of 4.1 nM as compared to 3.9 nM for fulvestrant, and it effectively downregulates ER α (IC₅₀=12.7 nM) in both tamoxifen-sensitive (T47D) and tamoxifen-resistant (T47D/PKCa) breast cancer cells. It acts as an antiestrogen that exerts potent antiproliferative effects on tamoxifen-resistant breast cancer cells (MCF-7/TamR, T47D/PKCa, and T47D/Y537S). When orally administered to mice, rats, and Beagle dogs, ZB716 demonstrates superior oral bioavailability in all animal-based pharmacokinetic studies when compared to fulvestrant administered by subcutaneous injection. More importantly, orally administered ZB716 was found to potently inhibit xenograft tumor growth in mice. These pre-clinical data strongly suggest that ZB716 is a promising SERD drug that could offer significant improvement over existing SERD regimen of Faslodex. ZB716 is being prepared in an IND application for phase 1 clinical trial in ER+, HER2- advanced breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-02

Title: True effect of aromatase inhibitor (AI) treatment on global gene expression (expr) changes in postmenopausal ER+ breast cancer (BC) patients: A POETIC study (CRUK/07/015)

Gao Q, López-Knowles E, Cheang MCU Chon U, Morden J, Martin L-A, Sidhu K, Evans D, Martins V, Dodson A, Skene A, Holcombe C C, Mallon E, Abigail E, Bliss J, Robertson J, Smith I and Dowsett M. The Institute of Cancer Research, London, United Kingdom; The Royal Marsden Hospital, London, United Kingdom; Royal Bournemouth Hospital, Bournemouth, United Kingdom; Royal Liverpool University Hospital, Liverpool, United Kingdom; Queen Elizabeth University Hospital Glasgow, Govan, United Kingdom; Poole Hospital NHS Foundation Trust, Dorset, United Kingdom and University of Nottingham, Derby, United Kingdom.

Body: BACKGROUND Gene expression (expr) analyses are increasingly used for characterising the pharmacodynamic response of primary BC. This includes assessing ER+ BC's dependence on estrogen (E) by measuring gene expr changes after AI-treatment. However, differences in tissue sampling and other preanalytic procedures between samples taken at diagnosis (D) and surgery (S), may lead to systematic artifactual changes that are falsely ascribed to the intervention. To identify genes whose expr is truly affected by AI, we measured global gene expr changes from paired core-cut biopsies at D and S from patients in the POETIC presurgical window trial.

METHODS In POETIC, 4486 postmenopausal women with primary ER+ BC were randomised 2:1 to receive perioperative AI (2 weeks pre + 2 weeks post surgery, termed Tr) or no perioperative treatment (termed Con), allowing gene expr changes to be compared between Tr and Con. RNA was extracted from paired RNA-later stored core-cuts of 56 Con and 157 Tr patients and arrayed on Illumina whole genome expr BeadChips. Raw data was extracted, transformed, normalised and batch-corrected. Probes not detected ($p > 0.01$) in $\geq 25\%$ of samples were discarded. Impact of AI on genes was evaluated based on difference of the expr mean changes ($\log_2(S/D)$) of the Tr and Con samples.

RESULTS In the Con group, expr of 73 genes significantly changed ($FDR < 5\%$); 70 of these changed by a similar magnitude in the Tr group, indicating their change was independent of AI therapy but would have been artifactually discovered as changed by AI in the absence of Con. The 8 genes most up-regulated in Tr were all among the 20 genes most up-regulated in Con: many were early-response or stress-associated genes. Three of the 8 most down-regulated in AI were the most down-regulated in Con: all were haemoglobin-related. Expr of some genes was changed in Con (eg *MYC* increase) but was unaffected in Tr. Such artifactual gene changes in Con tumors conceal true AI-induced changes that would not be detected in the absence of comparison with Con.

615 genes were down-regulated and 472 up-regulated in Tr but not Con. The majority of down-regulated genes were cell cycle or proliferation-associated or E-regulated, including *ESR1*, *PDZK1*, *GREB1*, *HSPB1*. Functional mapping showed changes in the regulation of cyclins and cyclin dependent kinases impacting on G1/S and G2/M. Of note, up-regulated genes included *CDK6* (target for CDK4/6 inhibitors) and *CCND2*, involved in G1/S checkpoint regulation; *SNAI2*, *TGFB3*, *TGFBR2*, associated with tumour invasion and metastasis; and other genes involved in aryl hydrocarbon receptor, Glioblastoma Multiforme, HIPPO and p53 signalling.

CONCLUSION Expr of certain genes is altered by processes involved in presurgical window studies. In the absence of a Con group, these may be wrongly ascribed to an experimental intervention or wrongly considered as unaffected by the intervention (eg *MYC* in this study).

Down-regulation of E-responsive and proliferation genes was an expected response to AI but increased expr of genes such as *SNAI2*, *CCND2* and *CDK6* indicates immediate tumour re-wiring and provides mechanistic support for benefit from combination therapy with a CDK4/6 inhibitor.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-03

Title: Long-term comparison of anastrozole versus tamoxifen: Results from LATTE/ATAC

Cuzick J, Sestak I, Bianco A, Strobbe L, Bergh J, Hanusch C, Neven P, Dowsett M, Forbes JF F, Buzdar A, Smith R and Howell A. Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, QMUL, London, United Kingdom; University of Naples, Naples, Italy; Canisius Wilhemina Hospital, Nijmegen, Netherlands; Karolinska Institutet and University Hospital, Stockholm, Sweden; Frauenklinik vom Rotkreuzklinikum München, Munich, Germany; UZ Leuven, Leuven, Belgium; Institute of Cancer Research, London, United Kingdom; Calvary Mater Hospital, Newcastle, Australia; MD Anderson, Houston; American Cancer Society, Atlanta and University of Manchester, Manchester, United Kingdom.

Body: Background: Previous reports from the Anastrozole Tamoxifen Alone or in Combination (ATAC) trial have shown significantly prolonged disease-free survival, lower rates of recurrence and distant recurrence, and reduced contralateral breast cancer in patients treated with anastrozole compared to tamoxifen (Cuzick et al., Lancet, 2010). Here, we compare the long-term effects of anastrozole versus tamoxifen in patients randomised to either monotherapy arm in the ATAC trial.

Methods: Postmenopausal women with hormone receptor positive breast cancer randomised to anastrozole or tamoxifen in the main ATAC trial were eligible for the LATTE observational study. The primary objective was to compare the long-term effects of tamoxifen and anastrozole on time to recurrence and death beyond 10 years after randomisation. Secondary objectives included time to distant recurrence, cancer-specific survival, new breast primaries, other cancers, fractures, and cardiac/cerebrovascular events. Cox proportional hazard methods were used to compute hazard ratios (95% CI) for recurrence from the time of last publication (10 years median follow-up).

Results: 2452 women from 11 countries were entered into the LATTE study. 40 women withdrew consent and 759 women died or had a recurrence within 10 years, which left 1653 women for analysis (838 anastrozole vs. 815 tamoxifen). A total of 118 breast events (69 anastrozole (8.2%) vs. 49 tamoxifen (6.0%)) were reported. No significant difference between the two treatment arms were observed (HR=1.36 (0.94-1.97), P=0.098). 57 women had a distant recurrence (33 (3.9%) vs. 24 (2.9%)), 41 reported a loco-regional recurrence (23 (2.7%) vs. 18 (2.2%)), and 26 contra-lateral breast cancer were recorded (17 (2.0%) vs. 9 (1.1%)). None of the treatment comparisons were statistically significant. 305 deaths were recorded (147 (17.5%) vs. 158 (19.4%)), of which 31 were due to breast cancer. Significantly fewer gynaecological cancers were recorded with anastrozole (7 vs. 16; OR=0.42 (0.15-1.09), P=0.05), but overall the effect on other cancers was not significant (54 (6.4%) vs. 64 (7.9%)). Fractures, cardiovascular, and cerebrovascular events were evenly distributed between the treatment arms.

Conclusions: Although anastrozole was associated with significant fewer recurrences compared to tamoxifen in the first 10 years of follow-up, in this analysis, with limited number of patients, we could not find a significant difference between the two treatment arms.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-05

Title: 12 years' median follow up (MFU) of BIG 1-98: Adjuvant letrozole, tamoxifen and their sequence for postmenopausal women with endocrine responsive early breast cancer

Thürlimann B, Giobbie-Hurder A, Colleoni M, Jensen M-B, Ejlertsen B, de Azambuja E, Neven P, Láng I, Gladiëff L, Bonnefoi H, Harvey VJ J, Spazzapan S, Tondini C, Price K, Piccart-Gebhart M, Regan MM M, Gelber RD D, Coates AS S and Goldhirsch A. International Breast Cancer Study Group and BIG 1-98 Collaborative Group.

Body: Background

The Breast International Group (BIG) 1-98 study is a randomized, phase 3, double-blind trial that compared five yrs of adjuvant treatment with letrozole, tamoxifen, or their sequence in postmenopausal women with hormone-receptor–positive early breast cancer. The study is conducted by the International Breast Cancer Study Group (IBCSG) on behalf of BIG. 8010 patients (pts) were enrolled between March 1998 and May 2003, and first results demonstrating a significant DFS benefit favoring letrozole compared with tamoxifen were reported in 2005 at 25.8 months' MFU. Subsequent updates showed continuing DFS benefit and updated results published in 2011 at 8.1 yrs' MFU showed OS benefit. Industry-sponsorship of the original BIG 1-98 ended in 2010; IBCSG launched an observational, non-interventional long-term follow-up study (BIG 1-98 LTFU) to collect survival, disease status and adverse events for an additional 5 yrs. We report results from BIG 1-98 LTFU at 12 yrs' MFU.

Methods

The original trial includes the 8010 patients enrolled. The potential BIG 1-98 LTFU cohort consisted of 148 academic medical centers with a maximum of 6843 pts who were alive and continuing follow-up when the original study ended. Response bias was addressed using weighting class adjustments estimated using multivariable logistic regression. Unadjusted incidence rates are reported here per 1000 pt-yrs with 95% Poisson confidence intervals. An updated abstract will include adjusted incidence rates, as well as estimates of OS and DFS based on a weighted Kaplan-Meier approach. The database will close in July 2016.

Results

As of May 2016, 81 centers participated in the BIG 1-98 LTFU study, contributing data from approximately 3900 pts (57%) and extending MFU to 12 yrs. Compared with the potential cohort of 6843 pts, the ~3900 in the LTFU analytic cohort were more likely to be under age 65 yrs at enrollment, have node-positive disease, and have tumors that were < 2 cm, PgR positive ($\geq 1\%$), and with no evidence of peritumoral vascular invasion. Extended adjuvant endocrine therapy for primary BC was continued in 2% of pts. Unadjusted incidence estimates of myocardial infarction increased during LTFU, while incidence of thromboembolic events and osteoporosis decreased (Table). Variations in incidence rates were noted depending on recording mechanism (e.g. registry, clinic visit, telephone, information from family).

Adverse Event	Unadjusted Incidence Rate/1000 pt-yrs (95% CI)	
	During original study	During LTFU
Myocardial Infarction	1.7 (1.4-2.0)	3.5 (2.7-4.5)
Thromboembolic event	6.0 (5.4-6.6)	2.5 (1.8-3.3)
Osteoporosis	23.6 (22.5-24.9)	18.2 (16.3-20.3)
Bone fractures	17.2 (16.2-18.3)	15.0 (13.2-16.9)

Overall 1845 deaths were reported; the unadjusted incidence of death was lower in the original study compared with during LTFU (21.9 vs. 26.6/1000 pt-yrs); incidence remained relatively stable for pts assigned to tamoxifen (24.9 vs. 25.2/1000 pt-yrs), and increased for pts assigned to letrozole (22.0 vs. 27.1/1000 pt-yrs).

Conclusions

The BIG 1-98 LTFU study has been successfully conducted. The additional data from the BIG 1-98 LTFU study provides important long-term clinical information about OS, DFS and adverse events.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-06

Title: Addition of ovarian function suppression to endocrine therapy in premenopausal women with early breast cancer: A meta-analysis

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Body: Introduction

The Suppression of Ovarian Function Trial (SOFT) and the Tamoxifen and EXemestane Trial (TEXT), together with the Austrian Breast Cancer Study Group (ABCSG-12) and the Eastern Cooperative Oncology Group 3193 (E-3193 trials), have examined adding ovarian function suppression (OFS) using gonadotropin-releasing hormone (GnRH) agonists to endocrine therapy in premenopausal women with early stage breast cancer. However, OFS with GnRH agonist plus aromatase inhibitor has subsequently been found to not consistently reduce estradiol levels to optimal target in the SOFT-EST study (JCO 2016; 34:1584) and implications of these study findings for clinical practice are controversial. Therefore, to further examine this issue, we conducted a systematic literature review and meta-analysis of available evidence.

Methods

We updated a recently published literature review of randomized clinical trials (JCO 2016; 34:1689) through June 1, 2016 using the keywords “breast cancer,” “ovarian function suppression,” “tamoxifen,” and “aromatase inhibitor”. Of 683 records identified through database searching, the 4 studies named above met inclusion criteria and were included in the quantitative synthesis. Information on disease-free survival (DFS) and overall survival was examined using hazard ratio (HR) and 95% confidence interval (CI). Because of significant heterogeneity in one performed meta-analysis, the random-effects (DerSimonian and Laird) method was used to estimate the combined RR for studies (Open MetaAnalyst).

Results

Combining ABCSG-12, SOFT and TEXT trial findings, OFS addition to aromatase inhibitor resulted in 65 fewer DFS events across the 3 trials compared to OFS addition to tamoxifen (350 DFS events vs. 415 DFS events, respectively, HR 0.89, 95% CI 0.57-1.39, $P=0.62$, $\text{Tau}^2=0.09$, heterogeneity $P<0.01$). In contrast, 30 more deaths were seen with OFS plus aromatase inhibitor use (155 deaths vs. 125 deaths, respectively, HR 1.31, 95% CI 0.93 -1.84, $P=0.12$, $\text{Tau}^2=0.03$, heterogeneity $P=0.18$). Combining SOFT and E-3193 trials to compare OFS addition to tamoxifen to tamoxifen alone use, more concordance between DFS events and overall survival is seen. There were 24 fewer DFS events across the 2 trials with OFS addition to tamoxifen compared to tamoxifen alone (160 DFS events vs. 184 DFS events, respectively, HR 0.83, 95% CI 0.67-1.03, $P=0.09$, $\text{Tau}^2=0$, heterogeneity $P=0.94$) and there were 14 fewer deaths in the OFS plus tamoxifen compared to tamoxifen alone group (58 deaths vs. 72 deaths, respectively, HR 0.76 95% CI 0.53 -1.07, $P=0.12$, $\text{Tau}^2=0$, heterogeneity $P=0.78$).

Conclusion

In conclusion, the apparent discordance between DFS and overall survival in the aromatase inhibitor and OFS trials and the results suggesting incomplete and/or intermittent estrogen suppression with GNRH analogs in the SOFT-EST trial suggest that adoption of OFS plus aromatase inhibitor use as adjuvant therapy in premenopausal women with early stage breast cancer may be premature. Longer term survival analyses of the endocrine therapy trials are needed before reliable risks and benefits of aromatase inhibitor plus OFS and tamoxifen plus OFS use as adjuvant therapy can be determined.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-07

Title: Drugs don't work if people don't take them: Non-initiation of endocrine therapy in young women

Rosenberg SM M, Gelber S, Ruddy KJ J, Tamimi RM M, Schapira L, Borges VF F, Come S, Meyer ME E and Partridge AH H. Dana-Farber Cancer Institute, Boston, MA; Mayo Clinic, Rochester, MN; Brigham and Women's Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA; University of Colorado Cancer Center, Denver, CO and Beth Israel Deaconess Medical Center, Boston, MA.

Body: Background: Despite the well-established survival benefit associated with adjuvant hormonal treatment, younger women with hormone receptor positive (HR+) breast cancer (BC) are less adherent to endocrine therapy (ET) as prescribed, compared to their older counterparts, and may have unique issues that contribute to ET non-adherence. In an effort to identify factors that can be targeted to improve ET uptake and adherence, we sought to evaluate ET initiation in young women with BC.

Methods: As part of a multi-center, prospective cohort enrolling women with newly diagnosed BC at age ≤ 40 years between 2006-2016, we identified 657 women with HR+, Stage 0-III BC. Participants complete serial surveys that included questions about socio-demographics, fertility concerns, and treatment. Women who did not report taking tamoxifen or an aromatase inhibitor (AI) at least once in the 18 months after diagnosis (dx) were classified as non-initiators. Variables significant at $p < 0.20$ in univariable models were entered into a multivariable logistic regression model to identify independent ($p < 0.05$) predictors of non-initiation.

Results: By 18 months post-dx, 15% (99/657) had not initiated ET; among women with Stage 0 BC, 77% (51/66) had not initiated vs 8% (48/591) with invasive BC ($p < 0.0001$). Among initiators (N=558), 93% were on tamoxifen, with 7% reporting AI alone or in addition to tamoxifen; 97% started ET within 12 months of dx. Among women with invasive BC (Table), non-initiation was associated with having less than a college degree (OR: 2.49 95% CI: 1.25-4.94), non-Caucasian race (OR: 2.58 95% CI: 1.18-5.64) and non-receipt of radiation (OR: 0.40 95% CI: 0.21-0.75). Age at dx, marital status, having children, employment, perceived financial comfort, fertility concern, stage, surgery, and chemotherapy were not significantly associated with non-initiation.

Conclusion: Most young women with HR+ DCIS do not take adjuvant ET despite the potential benefits (substantially reduced risk of local recurrence and contralateral BC) and very low risk of serious toxicity. Among young women with invasive HR+ BC, a significant minority fails to start ET within 18 months of dx. Adjuvant ET non-initiation may contribute in part to the racial and SES outcomes disparities that have been observed. Further study is needed to elucidate barriers to initiation with the goal of developing targeted interventions that will enhance ET initiation and adherence in general.

Table. Predictors of ET non-initiation in women with invasive BC (N=577*)

		Univariable		Multivariable	
	Median (range)	OR (95% CI)	p	OR (95% CI)	p
Age at dx (years)	36 (17-40)	1.08 (0.99-1.18)	0.07		
	N (%)				
Financial comfort	300 (51)	0.88 (0.49-1.60)	0.68		
Non-white	59 (10)	2.68 (1.25-5.71)	0.01	2.58 (1.18-5.64)	0.02
Full employment	282 (48)	0.76 (0.42-1.39)	0.38		
Less than college graduate	95 (16)	2.61 (1.36-5.02)	0.004	2.49 (1.25-4.94)	0.009
Married/living as married	452 (77)	0.79 (0.41-1.55)	0.50		
Children pre-diagnosis	369 (62)	1.11 (0.60-2.05)	0.75		
Fertility concern	234 (40)	0.92 (0.50-1.70)	0.80		
Stage (ref=3)					
1	244 (41)	1.22 (0.47-3.15)	0.68		

2	259 (44)	1.27 (0.50-3.24)	0.62		
Mastectomy vs lumpectomy	335 (57)	1.16 (0.64-2.12)	0.63		
Radiation therapy	331 (56)	0.40 (0.21-0.73)	0.003	0.40 (0.21-0.75)	0.004
Chemotherapy	411 (70)	0.66 (0.36-1.22)	0.18		

*multivariable analytic cohort

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-08

Title: Safety assessment of extended adjuvant endocrine therapy with letrozole; results of the randomized phase III IDEAL trial (BOOG 2006-05)

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Body: The implementation of adjuvant aromatase inhibitors (AI) has contributed significantly to an ongoing improvement in survival of early breast cancer patients, but the impact of extended AI on toxicity after use in the initial 5 years has not yet been studied. Earlier, it was established that even after 5 years of adjuvant tamoxifen, extended letrozole for 5 years was comparable to the earlier reported toxicities, without an effect on overall quality of life. The aim of the current study is to assess the reported toxicity in the IDEAL trial, investigating extended letrozole after 5 years of endocrine therapy.

The Dutch phase III IDEAL trial was designed to study the optimal duration of extended adjuvant letrozole, by randomizing to either 2.5 or 5 years of letrozole after the completion of 5 years of any endocrine therapy. All patients were disease-free at the time of randomization. The primary outcome of this study, disease free survival after extended endocrine therapy, will be reported separately. Toxicity data were collected every 6 months in the first year, and annually thereafter until study completion.

1824 patients were included in the trial, of which 26 patients never started with therapy (n=1798). In total, 3434 adverse events were reported by 1283 patients, causing 360 (20%) patients to stop therapy due to these events. In total, 643 patients randomized to 5 years of therapy reported 1834 AEs (71.2%), whereas 640 patients on 2.5 years of therapy reported 1587 events (71.5%). In the 5 year AI group, 1456 (80%) of the AEs were reported within the initial 2.5 years causing 177 patients to stop therapy in this period, versus 29 patients who stopped due to toxicity after the therapy extension beyond 2.5 years. There was no significant difference in the proportion of grade 3/4 events between both groups (2.5yr: 9.8%, 5yr: 9.7%, X^2 p=0.52). Most reported AEs were arthralgia (n=248, 13.8% of all patients, 8.9% grade 3/4), hot flushes (n=215, 12%, 6.5% grade 3/4), osteoporosis (n=181, 10%, 2.2% grade 3/4), fatigue (n=156, 8.7%, 5.1% grade 3/4) and a decrease in joint function (n=114, 6.3%, 3.5% grade 3/4). In total, only one grade 5 AE was reported, which was unrelated to the treatment (bleeding and sepsis after cholecystectomy).

Although the toxicity pattern is comparable to regular AI based adjuvant therapy, the percentage of specific adverse events like arthralgia and hot flushes is lower than expected. This observation is possibly a selection bias, since patients who encountered side effects during regular adjuvant therapy could have been less willing to participate in this trial. Remarkably, low numbers of AEs and therapy refusal due to toxicity were reported after therapy extension beyond 2.5 years. In conclusion, extended adjuvant endocrine therapy shows an acceptable toxicity pattern, and therapy extension beyond 2.5 years up to 5 years is well tolerated and safe.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-09

Title: The effects of treatment-induced symptoms, depression and age on sexuality in premenopausal women with early breast cancer receiving adjuvant endocrine therapy

Ribi K, Luo W, Burstein HJ J, Naughton MJ J, Chirgwin J, Ansari RH H, Walley BA A, Salim M, van der Westhuizen A, Abdi E, Francis PA A, Budman DR R, Kennecke H, Harvey VJ J, Giobbie-Hurder A, Fleming GF F, Pagani O, Regan MM M and Bernhard J. International Breast Cancer Study Group (Switzerland) and North American Breast Cancer Group (USA).

Body: Background: In premenopausal women with breast cancer any treatment that causes abrupt, premature ovarian failure increases the risk of sexual problems. Randomized-controlled trials in this population reported a worsening in sexual functioning over time irrespective of adjuvant endocrine treatment. We investigated key symptoms related to endocrine therapy, depression and age as predictors of sexual problems in premenopausal women with early breast cancer treated in the IBCSG TEXT/SOFT trials over the first two years of endocrine therapy.

Methods: A subset of patients (pts) enrolled by centers with English as primary language to TEXT (1027 of 2672 pts) and SOFT (1260 of 3066 pts) completed a questionnaire consisting of global and symptom-specific quality of life indicators, the CES-Depression (CES-D) and the MOS- Sexual Problems (MOS-SP) measures at baseline, 6, 12 and 24 months. The analysis considered 5 cohorts of pts according to chemotherapy use (yes/no), trial (SOFT/TEXT) and endocrine treatment assignment (tamoxifen alone [T], T or exemestane [E] with ovarian function suppression [OFS]). Mixed modeling was used to test the effect of the following on changes in sexual problems (MOS-SP total score) over two years: changes in treatment-induced symptoms (hot flushes, vaginal dryness, sleep disturbances, bone/joint pain, troubled by weight gain, tiredness, nausea/vomiting) from baseline to 6 months; depression at 6 months; and age at randomization. The model included severity groups of symptoms, depression (all dichotomized by median) and age (< 40 vs ≥40 years), 5 cohorts, time points (6, 12, 24 months), baseline covariates, and interactions of symptoms, timepoints and cohorts.

Results: Overall across cohorts, pts with more severe worsening of vaginal dryness and sleep disturbances at 6 months reported a greater increase in sexual problems at all timepoints ($p < .0001$). The effect of vaginal dryness on sexual problems was most pronounced in the cohort of pts who received T+OFS or E+OFS without chemotherapy; the effect of sleep disturbances was most pronounced in the cohort with prior chemotherapy and T alone. All other symptoms had a smaller impact on differences in changes of sexual problems. Significant effects were only seen in the short-term and varied according to cohort. Severity of depression at six months did not predict sexual problems at the later timepoints in the overall population. In the cohort that received T+OFS or E+OFS without chemotherapy, pts who had more severe depression scores at 6 months reported significantly worse sexual problems at all timepoints ($p < .05$). No differences were found for younger vs. older pts with respect to sexual problems at any timepoint.

Conclusion: Among several key symptoms related to endocrine therapy, only vaginal dryness and sleep disturbances significantly predicted sexual problems during the first two years in pts who received adjuvant endocrine therapy with or without chemotherapy. Depression predicted sexual problems only in the cohort of pts who received combined endocrine treatment without chemotherapy. Early identification of vaginal dryness, sleep disturbances and depression is important for timely and tailored interventions.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-10

Title: A new oral SERD AZD9496 for treatment of hormone dependent postmenopausal breast cancer

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Body: Treatment of hormone sensitive breast tumors with endocrine therapy such as antiestrogens or aromatase inhibitors has improved their clinical outcomes. However, not all tumors respond and the ones that do respond may eventually acquire resistance. One of the proposed mechanisms of resistance to endocrine therapy is overexpression of ER α and cross-talk of ER α with growth factor receptors. Studies including our own have shown that downregulation of ER with pure antiestrogen fulvestrant in combination with AIs may prolong responsiveness of the tumors to endocrine agents. Fulvestrant has been employed as either first or second line treatment for ER positive breast cancers alone or in combination with AIs. Studies have suggested that further escalation of dose may provide further benefit. However, dose escalation of fulvestrant which is administered via intramuscular injection is difficult due to its poor solubility. To overcome this shortcoming of an injectable drug, a novel orally active SERD (selective estrogen receptor downregulator), AZD9496 was developed. In addition to being orally active, AZD9496 is selective for mammary ER α . In the current study, we compared the effect of AZD9496 and fulvestrant on the growth of MCF-7Ca (human estrogen receptor positive MCF-7 cells stably transfected with human placental aromatase gene) xenografts grown in ovariectomized athymic nude mice. Tumors were allowed to form with androstenedione (aromatizable source of estrogen) supplement. When the tumors reached ~ 250 mm³, mice were grouped such that the mean tumor volumes were not significantly different ($p > 0.99$). Mice bearing xenografts of MCF-7Ca were then treated with fulvestrant (1 mg/d-*sc*) or AZD9496 (5 mg/kg/d-*po*), alone or in combination with anastrozole (200 μ g/d-*sc*) for 23 weeks. Tumors were measured weekly and growth rate was calculated. AZD9496 was significantly better at inhibiting the growth of tumors compared to control ($p < 0.001$) and anastrozole ($p = 0.04$). AZD9496 was equally effective in inhibiting the growth of MCF-7Ca xenografts as fulvestrant (growth rate, $p > 0.99$ and tumor volume on week 23, $p = 0.99$). In the second study, efficacy of AZD9496 was evaluated on against anastrozole resistant MCF-7Ca xenografts. Tumors were treated with anastrozole (200 μ g/d) for 13 weeks. During this time, the tumors initially regressed but eventually began to grow and had doubled in volume. At this time-point, they were regrouped to receive second line treatment. Single agent AZD9496 was marginally significant compared to continued anastrozole treatment ($p = 0.07$). Nevertheless, second line treatment with AZD9496 was equally effective as fulvestrant ($p = 0.36$). The combination of anastrozole with AZD9496/fulvestrant was more effective in reducing tumor growth compared to continued anastrozole treatment. Next, we measured the effect of AZD9496 on the mouse uterus. Uterine weight of mice treated with AZD9496 was not significantly different from mice that were treated with androstenedione ($p = 0.99$). These results suggest that AZD9496 was selective for tumor ER α and had no effect on the uterine ER α . These results suggest that AZD9496 may be a better alternative to fulvestrant due to its selectivity for mammary ER and same efficacy as fulvestrant obtained upon oral administration.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-11

Title: Metabolite-guided long-term prediction of outcome in tamoxifen treated breast cancer patients

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Body: Background

The genetic polymorphisms of CYP2D6 determine its enzymatic activity thereby the pharmacokinetic velocity by which tamoxifen (tam) is converted into active metabolites. Combined analyses of tam metabolites compared to CYP2D6 type and long-term survival could allow prediction of tam response and personalization of therapies. The clinical importance of defining such subgroups in the era of the new long-term (10-year) tam treatment paradigm is actualized.

Material and Methods

From May 1995 to December 1998, patients were included in an observational micro-metastasis study in the Oslo region and treated according to the national guidelines at the time (1). Serum samples were drawn at 3-year follow-up from 356 relapse-free patients, 106 of these were treated with tamoxifen. The median follow up time for breast cancer death was 16.8 years (3.5-19.4). Serum samples were processed using protein precipitation with acetonitrile. An Aquity UPLC system was used to chromatographically separate 10 tam metabolites using a BEH C18 Phenyl column (100 x 2.1 mm, 1.7 Åµm particle size) that was developed with a water-methanol gradient containing 0.1% formic acid. The LC system was coupled to a Xevo TQ-S tandem mass spectrometer equipped with an atmospheric pressure photoionization source. The method was validated with respect to linearity, imprecision, accuracy, and functional sensitivity according to FDA guidelines.

Results

The new LC-MS/MS method separated the active Z-isomers of 4OHTam and 4OHNDtam (endoxifen) from its inactive Z'-isomers and E-isomers. Imprecision (intra and inter-day CV %) was within 10 % for target concentrations for all metabolites and accuracies were in the range 95-106%. The method was validated with serum samples from 42 breast cancer patients using 20 mg of tamoxifen.

The endoxifen concentrations ranged from 0 to 90 nM, with a median value of 25 nM. The previous observed endoxifen level of 10 nM in poor metabolizers (2) was used as cut-off for the grouping of patients. The nil endoxifen (NE) group (< 0.1nM, n=14) or low-endoxifene (LE) group (0.1-10 nM, n=8) were grouped together. Univariate survival analysis did not show a significant association between breast cancer specific survival and endoxifen levels. (p=0.15; logrank and p=0.18;Breslow). However, for the period beyond 10-years of follow-up the breast cancer survival differed between the high endoxifene (HE) group and the NE+LE groups. For patients surviving the first 10 years the breast cancer specific survival was 94.2% vs. 77.8% for the HE and NE+LE groups respectively (p=0.020, logrank and p=0.017, Breslow, HR=4.5, CI 95=1.1-17.9). In the multivariate analysis endoxifen ≤/ > 10 nM remained the only factor in the final model.

Discussion

We developed a new accurate and precise LC-MS/MS method for the measurement of 10 tamoxifen metabolites. Importantly, the method separates active and inactive isomers of 4OHTam and 4OHNDtam/endoxifen. Despite the low number of patients, we observed a poorer long-term survival beyond 10 years in patients with nil or low serum concentration of endoxifen. A comprehensive analysis is presented addressing the relationship between genotyped based and metabolite based prediction of long-term outcome in tamoxifen treated breast cancer patients.

Ref:

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2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-12

Title: A single-blind, randomized, placebo-controlled phase II study to evaluate the impact of oral ibandronate on bone mineral density in osteopenic breast cancer patients receiving adjuvant aromatase inhibitors: Final results of the single-center BONADIUV trial

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Body: Background. Several randomized trials demonstrated aromatase inhibitors (AI) superiority in terms of disease-free survival compared to tamoxifen treatment for postmenopausal hormone receptor-positive breast cancer (BC) patients. Anyway AI toxicity profile due to estrogen suppression is a concern. Pivotal trials demonstrated a significant bone mineral density (BMD) loss due to AI, with a consistent risk of fractures, thus impacting on patients' quality of life.

Bisphosphonates represent an effective treatment in postmenopausal osteoporosis fractures prevention. Several studies demonstrated that upfront bisphosphonates therapy prevents bone loss in postmenopausal women receiving adjuvant AI for early-stage BC. However an adequate patients selection for adjuvant bisphosphonates treatment during AI endocrine therapy is still a challenge.

We present the final results of the BONADIUV trial, a single-blind, randomized, placebo-controlled phase 2 study designed to evaluate the impact of ibandronate treatment on BMD in osteopenic women taking AI.

Methods. Between January 2011 and May 2014, 561 patients underwent a baseline BMD assessment before starting AI as planned adjuvant treatment. Overall 171 osteopenic patients (lumbar spine [LS] and/or trochanter $-1 < T\text{-score} < -2.5$), were randomized in a 1:1 ratio to receive either placebo or oral monthly ibandronate (150 mg). All patients receive oral supplementation of calcium and vitamin D3. Study duration was 2 years. Exclusion criteria were: premenopausal status at time of randomization; comorbidities with increased risk of osteoporosis; body mass index < 18 ; chronic use of steroids; previous use of bisphosphonates; psychiatric disorders. Primary endpoint was the mean BMD difference between the two arms at a 2-year follow up. ClinicalTrials.gov identifier: NCT02616744. A total of 72 patients per arm of treatment were needed to obtain an 85% statistical power in order to detect a 2% BMD mean difference between the two arms. Considering a 10% dropout, at least 158 patients were required.

Results. A total of 171 patients were randomized in the study. Overall 27 patients (15.8%) withdrew the protocol (17 ibandronate vs 10 placebo arm): the final analysis was performed on 144 patients (72 patients per arm). P-value from Wilcoxon test showed no significant difference between arms at baseline both for LS ($p=0.94$) and trochanter ($p=0.83$).

At 2-year, osteopenic patients treated with ibandronate gained +18.7% and +15.5% at the LS and trochanter BMD, respectively. Patients treated with placebo lost -13.3% at the LS, and gained +2.9% at the trochanter.

Trochanter p-value from covariance analysis showed a mean BMD change significantly in favor of ibandronate arm at 1-year ($p=0.012$), and borderline at 2-year ($p=0.087$). Concerning LS, the mean BMD change was significantly in favor of ibandronate arm both at 1-year ($p=0.002$) and 2-year ($p<0.0001$).

Conclusions. Final results of our study showed that treatment with ibandronate, as compared to placebo, improved BMD change in osteopenic women treated with adjuvant AI, and consistently protected patients' bone loss.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-13

Title: Adverse events rates in a privately insured US population on tamoxifen compared to controls from the MarketScan^R database

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Body: Patients with breast cancer (BC) and those at high risk for BC are often reluctant to initiate Tamoxifen (Tam) due to potential adverse events (AEs) such as deep vein thrombosis (DVT), pulmonary embolus (PE), acute ischemic stroke (AIS), myocardial infarction (MI) and endometrial cancer (EC). Some decline this life saving therapy due to fear of AEs. AE rates from RCTs are low, but AE rates in the general population are not well documented.

Methods: The MarketScan^R database (2010-2013) was searched for patients aged 30-64 with a prescription of Tam active for more than 120 days. Patients with prior DVT, PE, AIS, MI or EC were excluded. ICD-9 codes for DVT, PE, AIS, MI and EC for inpatient and outpatient encounters were used to determine the frequency of AEs while on Tam. Patients taking Tam for prevention, DCIS or invasive cancer were included. Results were stratified by sex, age and time on Tam (in years). A random control group was created from the database utilizing patients age 30-64 with a levothyroxine prescription active for more than 120 days to calculate odds ratios (OR) for the AEs.

Results: 67,887 patients on Tam, including 1622 males, were analyzed, for a total of 93,498 Tam Exposure Years. The control group included 2.4 million patients. The average age of Tam users was 48 (males), 50 (females). The average age of controls was 51 (males), 50 (females). Ages (%) represented in Tam users: 30-34 (2%), 35-44 (20%), 45-54 (52%), 55-64 (26%). The average time on Tam was 1.4 years for females, 1.0 years for males (.33-4.2). DCIS accounted for 17% of Tam use, <1% was for prevention. AE rates and odds ratios (OR) are in the table below.

Adverse Events Rate and Odds Ratios

	AE% TAM users	AE% Controls	OR (95% CI)	AE% in 55-64 Year Olds	AE% in 30-54 Year Olds	OR Male v Female	OR >2 Yrs TAM use vs.< 2 Yrs TAM use
DVT	1.42	0.72	2.37 (2.22-2.53)	1.70	1.32	1.30	1.07
PE	0.87	0.43	2.37 (2.17-2.58)	1.12	0.78	1.33	1.12
AIS	0.51	0.70	0.89 (0.80-.099)	0.83	0.40	1.25	1.16
MI	0.37	0.70	0.80 (0.70-.090)	0.60	0.30	2.85	1.22
EC	0.90	0.29	3.03 (2.78-3.29)	1.52	0.68	N/A	0.99 *

*Not statistically significant

Conclusions: Despite an increased odds of DVT, PE and EC, AE rates in patients on Tam are remarkably low, especially in the younger age population, the population most likely to be on Tam. The odds of AIS and MI are lower in Tam users than controls. The favorable risk/benefit profile of this drug should be emphasized to patients who would benefit from its use, with the use of actual event rates, not odds ratios, being the focus of discussion with patients. Male sex increases the odds of DVT, PE, AIS and MI. Increasing age and use of Tamoxifen for more than 2 years increase the odds of having an AE. Further analysis will evaluate whether there are differences in AE rates between Tamoxifen patients based on their diagnosis (prevention, DCIS, invasive cancer).

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-14

Title: Association between BMI and residual estradiol levels in post-menopausal women using adjuvant letrozole: Results of a prospective study

Cescon DW W, Ennis M, Pritchard KI I, Townsley C, Warr D, Elser C, Rao L, Stambolic V, Sridhar S and Goodwin PJ J. Princess Margaret Cancer Centre, University Health Network, Toronto, Canada; Sunnybrook Odette Cancer Centre, ON, Canada; Lunenfeld Tanenbaum Research Institute, Toronto, Canada; Women's College Hospital, Toronto, Canada; Applied Statistician, Markham, Canada; Mount Sinai Hospital, Toronto, Canada and University of Toronto, Toronto, Canada.

Body: Background: Adjuvant aromatase inhibitors (AI) are the standard of care for post-menopausal women with early breast cancer (BC). Some studies have suggested that women with high BMI have less benefit from some AIs (anastrozole, but not letrozole) vs. tamoxifen. Of concern, a positive correlation between BMI and residual estradiol in women using letrozole has been reported (ALQUOT Study; JCO 2012). These findings have created uncertainty about the use of AIs in overweight and obese women. We conducted a prospective study to measure estrogen levels in post-menopausal women using adjuvant letrozole, with the primary objective of evaluating the relationship between residual E2 and BMI.

Methods: Post-menopausal women with early BC taking adjuvant letrozole for at least 3 months were recruited at 4 sites in Toronto, Canada. Fasting blood was collected 24 hours after the prior dose at baseline (Day 1, routine use of own letrozole, including generic) and on Day 29, following 28 days of monitored adherence to a provided supply of Femara 2.5 mg/day (Part A). Participants with BMI>25 were treated for a further 28 days (Part B) with a double dose of Femara (5 mg/day). Estradiol and estrone were measured using a high sensitivity liquid chromatography-tandem mass spectrometry assay. Vitamin D and markers of obesity/inflammation were assayed, and symptom/quality of life questionnaires completed at the same time points.

Results: 112 eligible patients were enrolled and completed Part A. Median age was 62 and BMI 24.7 kg/m² (range 19.0 to 42.2 kg/m²). 68% of participants had received adjuvant chemotherapy. Estradiol levels (mean±SD) were 2.81±1.15 pg/mL at baseline (typical use) and 2.69±1.01 pg/mL at Day 29 following monitored Femara use with near-perfect adherence (p=NS). No significant correlation was observed between estradiol and BMI on either Day 1 or Day 29 (r=0.06, p=NS). As previously reported, letrozole 5 mg/day dose did not affect residual estradiol levels at Day 58 for women with BMI>25. Residual estradiol was not correlated with vitamin D or markers of obesity/inflammation (insulin, leptin, CRP).

Conclusion: No association was observed between BMI and residual estradiol in women using letrozole (generic or Femara) at standard doses. These results are consistent with the absence of a treatment-by-BMI interaction in the BIG 1-98 trial and they provide reassurance that letrozole is an appropriate treatment in overweight and obese women.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-15

Title: A multi-institutional, prospective study of incorporating the genomic platform breast cancer index as a tool for decision-making regarding extension of adjuvant endocrine therapy

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Body: Background: Extending adjuvant endocrine therapy (AET) for hormone responsive breast cancer (HRBC) from 5 to 10 years is beneficial for many in preventing late relapse. Current decision-making regarding extension relies on a decision-making process that weighs non-personalized recurrence risks against risks and benefits of extended AET. The Breast Cancer Index (BCI, BioTheranostics Inc) has been validated to quantify the risk of late recurrence and to predict likelihood of benefit from AET extension based on an individual's tumor genomic profile. The purpose of this study was to conduct a multi-institutional study to prospectively assess the impact of BCI i) on provider's recommendation using the BCI results; 2) the confidence with decision-making; and 3) patient's satisfaction regarding extension of AET.

Methods: Patients with stage I-III HRBC treated at Yale Cancer Center and University of Pittsburgh Medical Center (UPMC), who had completed at least 3.5 years of AET were eligible. BCI was performed on FFPE samples from the original tumor sample (bioTheranostics Inc.). Patients and physicians completed pre- and post-test questionnaires examining preferences for extending AET, patients also completed anxiety and decision-conflict surveys.

Results: 140 patients [mean age 61, 80% postmenopausal, 73% stage I] were included. No extended AET was recommended for 35.3% patients' pre-testing. Reasons physicians did not recommend extended AET were perceived low risk of recurrence (87%), risk of osteoporosis (25%) and side effects (13%). Extended therapy was recommended for 65.7% patients pre-testing. Integration of BCI resulted in a change in physician treatment recommendation in 29% of patients. The recommendation for no extended AET rose to 48% and recommendation for extended AET dropped to 52% (OR=1.76 95% CI 1.08-2.85; p=.003). Of the recommendations that changed (N=41), the majority (73%) was for not extending endocrine therapy. However, 27% of recommendations were to extend endocrine therapy because of high risk or high likelihood of benefit results. More physicians felt strongly confident in their recommendation after the test result (26.4%) than before (9.3%) (OR= 3.5 95% CI 1.77-6.95; p<.0001). Satisfaction of decision increased in 23% of patients (OR=2.72 95% CI 1.66-4.46; p<.0001). Patient reported concerns including the cost, safety and benefit of extended AET decreased from pre- to post-testing (p=.025; p<.0001; p=.0012 respectively)

Conclusions: Overall, incorporation of BCI into clinical practice resulted in significant changes in physician recommendations regarding AET duration, with the majority of recommendations for no extended AET. Physicians reported increased confidence for their recommendation when incorporating the test result. There was also a significant increase in patient satisfaction and decrease in patient reported concerns regarding cost, safety and benefit of extended AET. The BCI is a tool that could be incorporated into decision-making algorithms to enhance physician confidence and patient satisfaction with recommendations for extending AET.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-09-16

Title: How population-based data complement trial data in the adjuvant endocrine treatment of ER+/HER2+ breast cancers

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Body: Background

This study is part of the Netherlands Breast Cancer Project, initiated to address research questions that are unlikely answered by future randomized controlled trials (RCT). Here we investigated whether aromatase inhibitors (AI) are superior over tamoxifen (TAM), in the treatment of Estrogen Receptor (ER) positive Human Epidermal growth factor Receptor 2 (HER2) positive breast cancer, using treatment and outcome data from the population based Netherlands Cancer Registry (NCR). RCTs showed superiority of AI over TAM in postmenopausal ER+/HER2+ patients while a (non-significant) suggestion for worse outcome was observed among premenopausal patients treated with AI in the SOFT/TEXT trial. Perimenopausal women were not considered in these trials.

Methods

Dutch women without a prior malignancy, diagnosed between 2005-2007 with an ER+/HER2+, endocrine treated, non-metastatic, invasive breast cancer, were identified through the NCR and followed until 2013. Since data on menopausal status were lacking, we used age at diagnosis as a proxy to categorize patients as premenopausal (≤ 45 years), perimenopausal (45-55 years) and postmenopausal (> 55 years). A time-dependent variable was calculated indicating whether AI treatment was given for $> 50\%$ (denoted AI treated) vs. $< 50\%$ (denoted TAM treated) of endocrine treatment duration. Recurrence-free survival (RFS) and overall survival (OS) were assessed using an extended Kaplan-Meier survival estimator and Cox proportional hazards regression. Hazard Ratios (HR) for the TAM/AI comparison, were adjusted for chemotherapy, trastuzumab, age at diagnosis, lymph node status, grade, clinical T stage, and ovarian ablation.

Results

We included 1158 patients: 326 pre-, 306 peri- and 526 postmenopausal. Of these, 229 received TAM and 929 AI. During follow-up, 239 RFS and 184 OS events were observed. In the TAM treated group, 56 RFS and 45 OS events were observed, in the AI treated group 183 RFS and 139 RFS events were observed respectively.

No differences in RFS were observed comparing AI to TAM treated patients in the premenopausal (HR 1.33; 95% CI 0.71-2.49; $P=0.378$) and postmenopausal (HR 0.84, 95%CI 0.54-1.32; $P=0.456$) group. However, perimenopausal patients benefitted significantly from AI compared with TAM (HR 0.50; 95% CI 0.27-0.95). Results were similar for OS: no significant benefit from AI when compared to TAM in pre- (HR 1.41; 95% CI 0.62-3.19; $P=0.408$) and postmenopausal (HR 0.75; 95% CI 0.47-1.22; $P=0.245$) patients while perimenopausal patients derived significant benefit from AI treatment (HR 0.42; 95% CI 0.20-0.85; $P=0.016$).

Conclusion

In this population based cohort study we observed superiority for AI over TAM in the treatment of ER+/HER2+ perimenopausal patients. Data were suggestive in favor of AI when compared to TAM for postmenopausal patients while an indication of worse outcome with AI was seen in premenopausal patients, consistent with results of the SOFT/TEXT trial. Although we used age as a proxy for menopausal status, our results are consistent with previous RCTs. Population based data may therefore provide a reliable source of information when new RCTs might not be feasible anymore.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-10-01

Title: Genomic profiling of residual ER+ breast cancers treated with prolonged neoadjuvant letrozole reveals novel alterations in clinically resistant tumors

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Body: Background: Approximately 20% of patients with early ER+ breast cancer (BC) treated with adjuvant antiestrogen therapy eventually relapse with endocrine-resistant metastatic disease. We hypothesized that profiling newly diagnosed ER+ BC that persist following prolonged estradiol deprivation with letrozole would identify genomic alterations associated with endocrine resistance.

Methods: We treated 57 postmenopausal women (median 77 years; range 60-86) with ER+/HER2- BC with neoadjuvant letrozole (median 7.5 months; range 3-36) followed by surgery and adjuvant endocrine therapy. Patients were followed with serial ultrasounds and defined as non-responders if they developed recurrent locally or metastatic disease, or had a preoperative endocrine prognostic index (PEPI) ≥ 4 (composite score of post-treatment ER, Ki67, T and N status). Post-treatment specimens were profiled by RNA-seq and targeted capture NGS of >300 cancer-related genes. We screened for variants with a high probability of disrupting protein function (GERP score >4) and excluded likely germline variants by filtering out every alteration not present in COSMIC, if the variant had an allele frequency >0.1% as per the ExAC dataset.

Results: Ten patients (17.5%) had a PEPI 0 score, 31 (54%) were PEPI 1-3, and 16 (28%) were PEPI ≥ 4 . After a median follow-up of 50 months (12-100), 9 patients (15.7%) had recurred with metastatic disease (4 with PEPI 1-3, 5 with PEPI ≥ 4). We identified 294 variants with a median coverage >250x (206 nonsynonymous, 21 nonsense, 58 indels, 8 splice site). Recurrent mutations included *PIK3CA* (38%), *KMT2C* (28%), *CDH1* (15%), *NF1* (12%), *TP53* (10%), *MAP3K1* (7%), *ERBB2* (7%) and *ESR1* (5%). Recurrent amplifications were identified in *MCL1* (31%), *GNAS* (19%), *CCND1* (16%), *FYN* (14%), *AURKA* (12%), and *ERBB2* (10%), while recurrent deletions were found in *DUSP4* (12%), *NCOR1* (8%) and *NF1* (6%). Compared to alterations reported in untreated ER+ breast cancers in TCGA, we observed a significant increase in *KMT2C*, *NF1*, *MCL1* and *FYN* alterations (FDR<0.05). *MCL1*, *GNAS* and *FYN* amplifications, *DUSP4* deletions, *MAP3K1* mutations, and *ERBB2* and *NF1* alterations were enriched in the non-responder group. Differential expression analysis of the RNA-seq data revealed an enrichment of E2F and MYC target genes, and genes involved in the G2/M checkpoint, TORC1 signaling, EMT and immunosuppression in non-responding tumors. PEPI 0 tumors were enriched with Luminal A subtype tumors, whereas PEPI 4 tumors were enriched with Luminal B, basal and HER2-enriched subtypes. Luminal A tumors exhibited improved disease free survival compared to other subtypes (HR 0.28, 95% CI 0.10-0.64). Gains in the proximal portion of chromosome 1q were associated with poor long-term outcomes as the relapse-free survival rate at 40 months for patients with 1q gains was 89% versus 41% for patients with no 1q gain (p=0.001).

Conclusions: Genomic profiling of residual ER+ breast cancers treated with prolonged neoadjuvant letrozole revealed a different mutational landscape than primary untreated ER+ BC. These alterations may be associated with poor response to estrogen deprivation in early breast cancer and deserve further study.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-10-02

Title: The impact of intrinsic subtypes and molecular features on aromatase inhibitor induced reduction of proliferation marker of Ki67 in primary ER+ breast cancer: A POETIC study (CRUK/07/015)

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Body: Background

Neoadjuvant endocrine therapy (NAE) is often a good option for postmenopausal (PM) women with estrogen receptor positive (ER+) breast cancers (BC). Fall in Ki67 is widely accepted as valid for predicting favorable tumor response to NAE and improved outcome. We report our planned correlative study to investigate if intrinsic subtype impacts on Ki67 changes (Δ Ki67) as measured by immunohistochemistry. We also explored the correlation of several ER+ BC relevant molecular features at baseline (B) with Δ Ki67.

Patients and methods

POETIC is a phase III, randomized 2:1 study for 4486 PM patients with ER+ BC to determine whether peri-operative aromatase inhibitor (AI) followed by standard adjuvant therapy improves outcome compared with standard adjuvant therapy alone. The proliferation rate was estimated as percentage (%) of cancer cells staining for Ki67. Primary biological endpoint was defined as two-week (2wk) change in Ki67 ($2wk\Delta Ki67$): $\ln[(2wk\ Ki67+0.1)/(B\ Ki67+0.1)]$. Secondary endpoint: "responders", was % change of Ki67 defined as $(2wk\ Ki67 - B\ Ki67) * 100 / B\ Ki67$. "Responder" was defined as follows: reduction <50% as poor (PR), 50-75% moderate and >75% as good responder (GR).

Human whole genome expression (GE) Illumina BeadChips were performed. Data was obtained from 137 paired samples from the treatment group (T) and 49 pairs from the control (C) group with GE data passing quality check and baseline Ki67 $\geq 5\%$ to minimise the impact of extreme values based on proportional Δ Ki67. Intrinsic subtype and risk of recurrence (ROR) groups were calculated using PAM50. GE scores from Oncotype Dx, MammaPrint, p53 mutation/wildtype (Troester 2006), ER+ early response (ERE) (Hatzis 2011), estrogen-regulated genes subtypes (Oh 2006) and markers for 23 different immune cell types (Bindea 2013) were calculated. Associations of GE scores to endpoints of response were determined by Spearman correlation and chi-square tests. Bonferroni correction was used to control error rate with $p < 0.0005$ deemed significant.

Results

At B of the 137 paired T, 64% were Luminal A (LumA), 22% Luminal B (LumB), 9% as HER-2 enriched (HER2-E), 2% as Basal-like (BLBC) and 3% as Normal-like. Subtypes at B were associated with response, with LumA showing the biggest reduction of Ki67 ($p=0.0001$) and GR. All GE, except ERE, correlated significantly with $2wk\Delta Ki67$ and response: higher risk groups associated with lowest reduction rate. None of immune cell types correlated with $2wk\Delta Ki67$, except that tumors enriched with T-helper 1 cell type were associated with PR ($p < 0.000001$).

Comparing subtypes between time-points, 85% of LumB and 42% of HER2-E were assigned instead as LumA at 2wk regardless of response. Of the 15 ROR defined high-risk group, only 33% were assigned instead as low-risk at 2wk.

Conclusion

Both LumA and LumB are endocrine sensitive. A fall of Ki67 was observed in majority of cases. Most tumors estimated as high-risk by molecular profiling showed less response and most remained moderate or high risk of recurrence on endocrine therapy. Whether molecular profiling at 2wk after starting AI predicts for long-term outcome in PM women with ER+ better than at diagnosis will need to be determined.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-10-04

Title: Using the 21-gene assay from core needle biopsies to choose neoadjuvant therapy for breast cancer: A multi-center trial

Bear HD D, Wan W, Robidoux A, Rubin P, Limentani S, White, Jr. RL L, Granfortuna J, Hopkins JO O, Oldham D, Rodriguez A and Sing AP P. Virginia Commonwealth University, Massey Cancer Center, Richmond, VA; Centre Hospitalier de l'Universite de Montreal, Montreal, QC, Canada; Cone Health Cancer Center, Greensboro, NC; Carolinas Medical Center, Charlotte, NC; Forsyth Regional Cancer Center, Winston-Salem, NC; Lynchburg Hematology Oncology Clinic, Lynchburg, VA; Methodist Hospital, Houston, TX and Genomic Health, Inc, Redwood City, CA.

Body: Neoadjuvant systemic therapy (NST) can facilitate breast conserving surgery (BCS) for large cancers. While hormone receptor positive (HR+) cancers respond to neoadjuvant chemotherapy (NCT), pathologic complete responses (pCR) are unlikely. Neoadjuvant hormonal therapy (NHT) may make BCS possible with less toxicity than NCT. We hypothesized that the Oncotype Dx® 21-gene Recurrence Score (RS), could guide the decision to treat with NHT versus NCT to facilitate BCS. This hypothesis is based on the ability of the RS to identify ER+ patients (pts) likely to benefit from adjuvant CT vs unlikely to benefit, as well as prior studies showing that pts with a low RS have no pCRs when receiving NCT (Yardley, et al 2015).

Methods: This prospective multi-center study enrolled pts with HR+, HER2-negative, invasive breast cancers not suitable for BCS (size ≥ 2 cm). Diagnosis was made by core needle biopsy (bx). Tissue blocks from the bx's were sent to Genomic Health for RS testing. Pts whose tumors had a RS < 11 were to receive NHT; pts with RS >25 tumors were to receive NCT; pts with midrange RS of 11-25 were randomized to NHT or NCT.

The primary objective was the feasibility of randomizing pts with RS values 11-25 between NHT and NCT. The primary endpoint was whether 1/3 or more of randomized pts would refuse assigned treatment. Secondary endpoints included: clinical partial and complete response (cPR, cCR) rates, overall clinical response rates (CR), pCR in the breast, pCR in the breast and nodes and successful BCS. One-sample binomial test was used to compare the observed refusal rate with 1/3, along with its 95% CI. Fisher's exact test, logistic regression (for a binary endpoint), and/or ordinal regression (for an ordinal endpoint) were used to compare the 4 treatment groups for secondary endpoints.

Results: Seven US and Canadian centers enrolled 64 pts; 5 were excluded (1 delay in RS result, 1 lost block, 1 HR testing discrepancy, 2 not eligible). Of 33 pts with RS 11-25, 5 (15%; 95% CI =2.9% - 27.4%) refused assignment to NCT (2 chose NHT and finished the study). This was significantly lower than the 33% target (binomial test, p=0.0292). Results for other endpoints are shown in the Table (according to treatment received); the total number of pts for the analyses is 55; 1 pt had missing data for clinical response.

Results According to Treatment

Treatment Group	RS<11 NHT	RS 11-25 NHT	RS 11-25 NCT	RS>25 NCT	Overall P
N	N=12	N=18	N=11	N=14	
cCR	8.3%	22.2%	36.4%	28.6%	0.0422
cPR	75%	27.8%	36.4%	64.3%	
CR (cCR + cPR)	83.3%	50%	72.7%	92.9%	0.0490
pCR Breast	8.3%	6%	0	21.4%	NS
pCR Breast + Nodes	0	0	0	14.3%	NS
Successful BCS	75%	72.2%	63.6%	57.1%	NS

Conclusions: This pilot showed the feasibility of using the RS to guide NST, with only a 15% refusal rate of randomly assigned treatment. Of greater interest is the finding that pts with a RS <11 had a high CR rate with NHT and that pts with a RS 11-25 who

received NHT had a similar rate of BCS success as the pts with RS <11. Conversely, pts with RS>25 treated with NCT had the highest CR (cCR + pCR) and pCR rates. These results demonstrate that conducting a similarly designed larger trial is feasible and suggests that for pts with a low RS, NHT is a potentially effective strategy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-10-05

Title: PowerPIINC trial: Changes in tumor proliferation index and quality of life with 7 days of preoperative tamoxifen

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Body: BACKGROUND: A decrease in Ki67 has been shown to be a predictor of response to tamoxifen. Previous trials have shown a decreased Ki67 proliferation index in breast tumors with as little as 2 weeks of preoperative tamoxifen. However, shortening the preoperative treatment time in window of opportunity studies increases patient acceptance for trial participation. The POWERPIINC trial examined the effect of 7 days of preoperative tamoxifen on breast tumor proliferation and patient symptoms.

METHODS: Adult women with untreated stage I or II invasive breast cancer that was ER positive (>1%) planning on breast surgery with no contraindications to tamoxifen were enrolled. Women received 20mg of tamoxifen for 7 days up to the day of surgery and for 14 days afterwards. Proliferation was assessed by Ki67 immunohistochemistry before and after 7 days of tamoxifen. The proliferation genes from the PAM50 were also assessed by RT-PCR. Symptoms and QOL were assessed by the FACT-ES, MENQoL, and BMQ.

RESULTS: 52 women were enrolled, and 44 were evaluable for Ki67. The median age was 58.5 years, and the median tumor diameter was 1.2cm. Most women (73%) were post-menopausal. Most tumors were PR positive (88%). Only 8% of tumors were HER2-positive. The Ki67 decreased by a geometric mean of 40% (95% CI 29%-63%), and 73% (95% CI 57%-85%) of women had tumors with decreased proliferation after 7 days of tamoxifen ($p=0.0001$ by paired t-test). No correlation was seen between the change in Ki-67 and change in FACT-ES or MENQoL scores. Women reported minimal to no bother from psychosocial or physical symptoms at baseline or on the day of surgery. Expression level of individual proliferation genes did not change after 7 days of tamoxifen.

CONCLUSION: Seven days of tamoxifen showed a similar relative decrease in the Ki67 proliferation index as that reported for longer courses. Therefore, short window of opportunity trials can be informative.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-10-06

Title: The long term outcomes of female patients treated with primary endocrine therapy for non-metastatic breast cancer

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Body: Introduction: Primary endocrine therapy is usually prescribed for elderly patients with breast cancer who are considered too frail for surgery. Many respond well but some patients progress requiring either a switch of treatment or, in some cases, surgical intervention for local disease control. This study aimed to look at all patients treated with primary endocrine therapy over a six year period with a particular focus on their survival and how many ultimately required to have surgery performed.

Methods: All female patients treated with primary endocrine therapy in our NHS board, diagnosed with breast cancer between August 2009 and July 2013, were identified from a prospectively collected database. Those with metastatic disease at diagnosis were excluded. Their progress and long term outcomes were detailed from each patient's electronic record.

Results: 171 patients were commenced on primary endocrine therapy for non-metastatic breast cancer during this period. The median age at diagnosis was 81 years (43-99 years). 132 of the tumours were ductal/no specific type, 22 were lobular, 7 mucinous and 10 were of other types. The median ER status was 8 (0-8). In all but two cases the agent used was an aromatase inhibitor. 65 (38%) patients were documented to have clinically and/or radiologically responded to therapy, 8 (5%) had static disease, 41 (24%) had disease progression and 47 (27%) were not followed up in clinic, usually as either a clinician decision based on their frailty or as the patient failed to attend appointments. In 10 cases, follow up appointments had been attended but there was no clear documentation regarding clinical progress. In those patients who had disease progression, the mean time to documented progression was 30 months (2-60 months). In the other patients who had follow up, mean length of follow up at the time of data collection was 27 months (2-84 months). 36 (21%) patients required a switch in endocrine therapy, in 31 cases due to disease progression and in the other 5, because of side effects. 13 (8%) patients ultimately required to have surgery. At the time of data collection, 104 patients had died with their mean survival being 537 days (4-2016).

Conclusions: While some patients treated with primary endocrine therapy for breast cancer had progressive disease requiring a switch of treatment or surgery, the majority of those followed up achieved disease control and tolerated the medication well. It therefore provides an acceptable alternative to surgery in the frail, elderly population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-11-01

Title: Non interventional study with netupitant/ palonosetron (NEPA) as CINV prophylaxis in highly or moderately emetogenic chemotherapy

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Body: Background

Nausea and Vomiting due to cancer therapy is still a problem for patients and physicians and therefore an ongoing item of research in oncology. International antiemetic guidelines (ASCO, NCCN, MASCC/ESMO) have been published and new drugs are introduced into the market. The fixed oral combination of the NK₁-receptor antagonist (RA) netupitant and the 5-HT₃-RA palonosetron (NEPA) was recently approved in US and EU for the prevention of acute and delayed chemotherapy-induced nausea and vomiting (CINV) in cancer patients receiving cisplatin-based highly emetogenic (HEC) or moderately emetogenic chemotherapy (MEC). The MASCC guidelines 2016 recommend a triple combination of 5-HT₃- and NK₁-RA and dexamethasone given on day 1 for patients receiving HEC, anthracycline / cyclophosphamide (AC)-containing chemotherapy as well as for carboplatin-based MEC for the prevention of chemotherapy-induced nausea and vomiting (CINV).

Objectives

The primary endpoint of this non interventional study is the evaluation of quality of life in adult cancer patients receiving NEPA for CINV prevention in MEC or HEC. Secondary endpoints are efficacy and safety of NEPA.

Methods

This non-interventional study evaluates CINV prophylaxis with NEPA and QoL in 2500 cancer patients receiving single day or two day MEC or HEC in an ambulatory setting in German cancer hospitals and specialized cancer practices. NEPA is prescribed in accordance with the EU marketing authorization. Quality of life is recorded by FLIE questionnaires. Efficacy - measured as complete response (CR, no vomiting, no rescue medication) –as well as additional medication, safety and adverse events (AEs) are documented by an online questionnaire filled by the physician and a patient diary. 3 consecutive chemotherapy cycles are documented online using the ODM QuaSi documentation system. All specifications in the online documentation must be verifiable.

Results

700 patients from 175 centers (93 gynaecologic oncology, 79 medical oncology, 3 urologic oncology) are included to date. The majority of patients were women (88.7%). 71% of all patients had breast cancer. 92.6% had an ECOG performance status of 0-1. 77.6% received (neo)adjuvant chemotherapy. Most common chemotherapy regimens were AC-based regimens (53.9%), carboplatin-based regimens (15.9%) and cisplatin-based regimens (12%).

Efficacy data are available for 486 patients in cycle 1 and 350 patients over 3 cycles. During 3 consecutive chemotherapy cycles, 89% of patients had a CR on day 1 as recorded by patient diaries. In the delayed phase (days 2-5), 85% of patients had a CR. 93% recorded no vomiting during the entire 5 days at risk following chemotherapy and 69% reported no or only mild nausea. > 90% of the medical staff rated the efficacy of the CINV-prophylaxis with NEPA as good or very good over 3 cycles.

Adverse events (AE), mostly constipation were rare and mild and only of grade 1 or 2. No serious AEs were observed.

Summary

NEPA is a safe and efficacious option for the prophylaxis of nausea and vomiting in highly and moderately emetogenic chemotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-11-02

Title: A longitudinal look at toxicity management within a platform trial: Lessons from the I-SPY 2 TRIAL

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Body: Background: I-SPY 2 is a multicenter, phase 2 trial using response-adaptive randomization within biomarker subtypes to evaluate a series of investigational agents or regimens when added to standard neoadjuvant therapy for women with high-risk stage II/III breast cancer - investigational agent (I) +paclitaxel (T) qwk, doxorubicin & cyclophosphamide (AC) q2-3 wk x 4 vs. T+/-HP/AC (control arm(s)). Although the primary endpoint is pathologic complete response (pCR) at surgery, a key secondary aim is to evaluate the toxicity profiles of these investigational agents. Distinct aspects of safety monitoring in a platform trial, as well as the specificities of safety management in a potentially curative population make the experiences from I-SPY 2 valuable to the community.

Methods: Inclusion and exclusion criteria are uniformly applied to all women in I-SPY 2. When a new investigational agent/regimen is planned for the trial, agent specific laboratory/hematologic limits or additional required tests are added, as needed. Eligibility criteria remain in the trial for its duration and apply to all investigational and control arms. Laboratory and adverse event data are collected and monitored in real time. The lead investigator of the investigational agent/regimen who chaperones a specific agent/regimen through the trial ("Agent Chaperone"), Medical Monitor, I-SPY 2 Agents Committee, CRO safety group, and an active DSMB that meets monthly oversee the management of toxicities within each investigational agent/regimen of the trial. Toxicity profiles for an investigational agent/regimen are compared to their relevant control. Safety analyses are intention to treat.

Results: From March 2010-May 2016, eleven (11) investigational agents/regimens have opened (and 6 have completed evaluation) and 973 women have been randomized. These agents/regimens span a variety of mechanisms of action including targeted therapies such as small molecule inhibitors and antibodies, as well as immunotherapies. Additions to the trial's eligibility criteria have been made with new investigational arms. Adverse events of special interest have been monitored for each investigational arm and specific toxicities treated uniformly when applicable. A risk-based monitoring plan has been implemented that focuses on the collection and review of the trial's most critical data elements including serious adverse events and drug specific safety issues, allowing for a more efficient and focused effort. Safety issues have been quickly addressed and requirements updated, when needed, given the importance of limiting (or avoiding) long-term safety complications within this neoadjuvant patient population. Accrual to the trial has (been) maintained over time and the safety of trial participants has been well managed.

Conclusion: A platform trial requires an evolving, and focused safety-monitoring process that adapts as new investigational agents are included. I-SPY 2's infrastructure and team science approach has created a system to manage patients across multiple arms with different risk profiles. These practices will support the safe evaluation of additional new combinations and regimens and serves as a guide for safety management within standing platform trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-11-03

Title: Incidence and severity of diarrhea with neratinib + intensive loperamide prophylaxis in patients (pts) with HER2+ early-stage breast cancer (EBC): Interim analysis from the multicenter, open-label, phase II control trial

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Body: Background: Neratinib (Puma Biotechnology Inc) is an irreversible pan-HER inhibitor in late-phase development for the treatment of early-stage and metastatic HER2+ BC. Diarrhea, the main toxicity of neratinib, requires active management with loperamide prophylaxis given early in the course of treatment. CONTROL (PUMA-NER-6201) is an international, open-label, phase II study investigating the efficacy of loperamide prophylaxis in the prevention of neratinib-associated diarrhea. CONTROL has recently been expanded to include prophylaxis with loperamide + budesonide, which targets inflammation identified in a preclinical model of neratinib induced diarrhea.

Methods: Pts with HER2+ early-stage BC who had completed trastuzumab-based adjuvant therapy were eligible. All pts were to receive oral neratinib 240 mg/day for 1 year + structured loperamide prophylaxis on d1–56 (2 cycles). Adverse events were graded according to NCI-CTCAE, v4.0. Primary endpoint: incidence of grade ≥ 3 diarrhea. A protocol defined interim analysis (data cut-off July 2016) was performed when ~120 pts had completed ≥ 2 cycles of neratinib + loperamide prophylaxis. A preliminary analysis of the loperamide + budesonide cohort was also performed at this time. Clinicaltrials.gov: NCT02400476.

Results: For the interim analysis, 133 pts received neratinib + loperamide prophylaxis. A further 16 (of 40 planned) pts received neratinib + loperamide prophylaxis (2 cycles) + budesonide (1 cycle). Key results are shown in the table. Incidence of grade ≥ 3 diarrhea was 27.1% with loperamide prophylaxis and 12.5% with loperamide + budesonide prophylaxis vs 39.9% without protocol-mandated loperamide prophylaxis (ExteNET). Grade 2 diarrhea also decreased (20.3%, 18.8% vs 32.5%, respectively). Grade 3 diarrhea events were uncommon after cycle 1 in all CONTROL cohorts.

Study	CONTROL				ExteNET ⁴
	Loperamide	Loperamide	Loperamide	Loperamide + budesonide	
Antidiarrheal prophylaxis	Loperamide	Loperamide	Loperamide	Loperamide + budesonide	Loperamide prn
Protocol	Original ¹	Modified ²	Cumulative (Original + Modified)	Amd 3 ^{2,3}	—
N (ITT at data cut-off)	28	105	133	16	1408
Diarrhea, %					
Any grade	82.1	69.5	72.2	68.8	95.4
Grade 2	25.0	19.0	20.3	18.8	32.5
Grade ≥ 3 ⁵	21.4	28.6	27.1	12.5	39.8
Grade 4	0	0	0	0	0.1
Median diarrhea episodes/pt					
Grade ≥ 2	2.0	1.0	1.0	1.0	3.0
Grade ≥ 3	1.0	1.0	1.0	1.5	2.0
Median time on study (neratinib), mo ⁶	9.7	3.7 ⁶	4.5 ⁶	1.0 ⁶	11.6

1. Oral loperamide 4 mg with first neratinib dose, then 2 mg q4h d1–3, then 2 mg q6–8h d4–56.
2. Oral loperamide 4 mg with first neratinib dose, then 4 mg tid d1–14, then 4 mg bid d15–56.
3. Oral budesonide 9 mg qd d1–28.
4. Analogous patient population [Chan et al. *Lancet Oncol* 2016].
5. Primary endpoint (CONTROL).
6. Cohorts are ongoing.

Conclusions: A structured loperamide prophylactic regimen for 2 cycles is associated with a lower incidence and severity of neratinib associated diarrhea, with notably less grade 2/3 diarrhea compared to ExteNET events. There appears to be some adaptation to the effects of neratinib, as higher-grade diarrhea occurs early and does not typically recur. Preliminary data suggest that adding budesonide may further improve outcomes; enrollment into the budesonide cohort continues.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-11-04

Title: Efficacy and safety of darbepoetin alfa or epoetin beta in 2994 high risk early breast cancer patients participating in the German adjuvant intergroup node-positive study (GAIN)

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Body: Background

Reduced quality of life during chemotherapy is often due to fatigue and dyspnea caused by chemotherapy-induced anemia. In breast cancer patients the incidence is estimated to be above 50% and rising during the course of treatment resulting as well in therapy delays. Despite red blood cell transfusion the administration of erythrocyte stimulating factors (ESF) serves as a treatment option. In comparison with conventional chemotherapy intense dose dense regimens have proven to be beneficial in high-risk breast cancer patients, but higher incidences of anemia have been reported. In the GAIN trial two dose dense regimens were evaluated and patients received either Darbepoetin alfa or Epoetin beta. The aim of this subanalysis was to analyse the efficacy and safety of the application of two different ESF during adjuvant chemotherapy for primary node positive breast cancer.

Methods

Patients were randomly assigned to receive three courses each of epirubicin (E), paclitaxel (T), cyclophosphamide (C) all given at 2-week intervals i.v. (idd ETC-regimen) or ddEC followed by paclitaxel weekly (Tw) plus capecitabine (X)(EC-TX-regimen). All patients received either primary prophylaxis with Epoetin beta (Epo) (450IE/kg weekly) or Darbepoetin alfa (D) (4,5µg/kg biweekly). Allocation happened alternately by date of randomization. Patient outcome (rate of anemia and thromboembolic events, disease free survival (DFS), overall survival (OS)), overall and by regimen were compared according to ESF type applied and in subgroups defined by age.

Results

2994 patients were randomized to receive one of the dose dense chemotherapies and of these 1482 patients were given Darbepoetin alfa and 1512 received Epoetin beta. In the trial 84.7% of patients suffered from anemia in the Darbepoetin as well as in the Epoetin group and grade 3/4 anemia was observed in 3.6% vs 3.1% of patients, respectively. In the ETC arm anemia rates, especially grade 3/4 were slightly higher, but there was no significant difference within the treatment arms according to ESF applied (anemia any grade: D 86.1% vs. Epo 87.2%; EC-TX: D 83.2% vs. Epo 82.3%). In the ETC arm anemia was most frequently observed in patients aged 60+ years, but there was no significant difference between the ESF (D 90.5% vs. Epo 89.8%). No significant differences in the incidence of anemia by ESF treatment were observed in various age groups of the EC-TX arm. Thromboembolic events occurred in 9.1% in the Darbepoetin group and in 10.1% of patients treated with Epoetin ($p=0.355$). Interestingly there were more thromboembolic events in the EC-TX arm compared to the ETC arm ($p<0.001$), but irrespective of the ESF type (EPC: D 7.0% vs. Epo 7.7%; EC-PX: D 11.3% vs. Epo 12.5%).

OS and DFS analyses showed no difference between ESF treatment overall as well as stratified by chemotherapy regimen.

Conclusion

High risk breast cancer patients treated with two different dose dense chemotherapy schedules have comparable incidences of anemia, thromboembolic events and a similar long term outcome if they receive a preventive treatment with either Darbepoetin alfa or Epoetin beta.

The trial is financially supported by Amgen and Roche.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-11-05

Title: Safety, immunogenicity and efficacy of proposed biosimilar pegfilgrastim (LA-EP2006) compared with reference pegfilgrastim in breast cancer: Pooled analysis of two randomized, double-blind, phase III trials

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Body: Background: Biosimilars are highly similar to a biological reference product with no clinically meaningful differences in terms of efficacy and safety. Here we present the pooled analysis of two randomized trials (PROTECT1 and 2) comparing the efficacy, safety and immunogenicity of proposed biosimilar pegfilgrastim (LA-EP2006) with reference pegfilgrastim (Neulasta®).

Methods: Two multinational, independent, prospective, double-blind, phase III studies (EudraCT: 2011-004532-58; 2012-002039-28) enrolled adult chemotherapy-naïve women with breast cancer scheduled to receive ≤6 cycles of (neo)-adjuvant chemotherapy with docetaxel 75 mg/m², doxorubicin 50 mg/m² and cyclophosphamide 500 mg/m² (TAC). Patients were randomized to receive a single 6 mg injection of LA-EP2006 or reference on Day 2 of each cycle. Primary endpoint was duration of severe neutropenia (DSN) (number of consecutive days with ANC <0.5×10⁹/L) in Cycle 1. Equivalence was confirmed if the 95% confidence intervals (CI) for the difference in mean DSN between groups were within a pre-defined margin of ±1 day. Secondary efficacy endpoints included incidences of febrile neutropenia (FN), fever and infections, and depth of ANC nadir and time to ANC recovery (≥2×10⁹/L after the nadir) in Cycle 1. Safety was assessed at each visit with follow-up visits at 4 weeks and 6 months (PROTECT1 only) after last administration of pegfilgrastim. Immunogenicity was assessed before the first pegfilgrastim injection, on Day 15 of cycle 6, and 4 weeks and 6 months (PROTECT1 only).

Results: A total of 624 patients were randomized (LA-EP2006: n=314; reference: n=310). Baseline demographics were well balanced (mean age: LA-EP2006: 49.3 years, reference: 49.8; median duration (months) since initial diagnosis: LA-EP2006: 1.33 [0.1–76.0], reference: 1.35 [0.2–11.2]; ECOG status 0: LA-EP2006: 78%, reference: 75%). Mean DSN difference in Cycle 1 was -0.04 days (95% CI: -0.19, 0.11), showing statistical equivalence. FN was reported in 5.7% of patients with LA-EP2006 vs. 8.4% with reference in Cycle 1 (all cycles: 8.0% vs. 10.3%). Across all cycles, frequency of fever (LA-EP2006: 18.5%; reference: 19.7%) and infections (LA-EP2006: 15.6%; reference: 18.1%) were similar in both groups. Mean ANC time courses were almost superimposable in the two groups, with similar time and depth of ANC nadir and median time to ANC recovery was 2 days in both groups in Cycle 1. Treatment-emergent adverse events (TEAEs) were similar across groups (LA-EP2006: 92%; reference: 89%), and TEAEs with a suspected relationship to pegfilgrastim were reported in 22.6% of patients with LA-EP2006 and 21.3% with reference across all cycles, with the most frequent being musculoskeletal and connective tissue disorders (LA-EP2006: 10.2%; reference: 9.7%). Serious TEAEs were reported in 14.3% (LA-EP2006) vs. 17.1% (reference) across all cycles. No neutralizing or clinically relevant anti-pegfilgrastim antibodies were identified.

Conclusions: LA-EP2006 demonstrated similar clinical efficacy and safety to reference pegfilgrastim in patients with breast cancer receiving myelotoxic chemotherapy.

*Neulasta® is a registered trademark of Amgen Inc.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-11-06

Title: HER2 positive breast cancer and subclinical cardiotoxicity by echocardiogram 2D Strain, Do chemotherapy sequence matter?

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Body: Background: Even when there are free schemes of anthracyclines, the biological subtype HER2+ can be exposed to cardiotoxic drugs anthracyclines (A) and/or trastuzumab(H). It has been demonstrated that the LVEF is not sensitive to detect early cardiac changes and could be within normal ranges but with irreversible cardiac dysfunction. 2D speckle tracking and cardiac biomarkers (TnI and NT-proBNP) have shown more sensitivity to define early myocardial damage. Adjuvant trial FinHER suggests lower cardiotoxicity (0.5%) when taxanes (T)+H are given before A. Our objectives were: 1)To show higher sensitivity of the longitudinal strain in the echo to detect cardiotoxicity compared to the LVEF 2)To describe if there are any differences in the cardiotoxicity in relation with the chemotherapy sequence used.

Patients and methods: A retrospective cohort of patients diagnosed with breast cancer from February 2008 to January 2016 was obtained, in which 46 patients with HER2 positive breast cancer were included, most in the context of neoadjuvant or adjuvant chemotherapy treated with sequential chemotherapy (A→T+H vs T+H→A), with a cumulative dose of 240 mg/m² of doxorubicin. A basal echo was done, as well at 3, 6, 9, 12 months after the beginning of the chemotherapy to determine LVEF and early cardiac damage parameters, such as longitudinal strain. Cardiotoxicity was determined with a symptomatic HF with a decline of LVEF >5% or LVEF ≤55% or asymptomatic HF with a decline ≥10% or in LVEF or LVEF ≤55%. A fall ≥15% was considered a significant decline of the longitudinal strain

Results: 1 patient (2.1%) presented symptomatic HF (NYHA III). Asymptomatic cardiotoxicity was present in 9 patients (19.6%), 9 (19.6%), 11 (23.9%), and 13 (28.3%) at the 3, 6, 9, and 12 months respectively. The mean LVEF were (66±6.6), (63±9.0), (65±7.0), (65±6.4), and (63±6.9) at the basal, 3, 6, 9, and 12 months measurements respectively, with a non-significant “p” value. Significant decline in the longitudinal strain was observed in 10 patients (21.7%, p=0.012), in 13 patients (28.3%, p=0.034), in 15 patients (32.6%, p=0.95), and in 18 patients (39.1%, p=0.07), at the 3, 6, 9, and 12 months measurements respectively. When comparing the cardiotoxicity group vs the non cardiotoxicity groups no significant differences were observed with other risk factors as: HTN, dyslipidemia, carbohydrate intolerance, DM, radiotherapy, weight, cumulative doses of A, systolic-diastolic BP, glc levels, HDL, TG, and BMI. In the logistic regression analysis the longitudinal strain decline (≥15%) basal vs 3 months measurement had a OR 7.63 CI 95%(1.04-55.86) and the A→T + H sequence OR 7.7 CI 95%(1.076-55.43), were kept as independent variables for cardiotoxicity.

Conclusions: As reported in literature the decline in the longitudinal strain was more sensitive than the LVEF to predict cardiotoxicity, thus this would allow to identify the highest risk individuals and to start a cardioprotective intervention. Due to the retrospective character of our study, we believe that in the neoadjuvant or adjuvant chemotherapy setting of sequential chemotherapy there is the need of prospective studies that evaluate the subclinical cardiotoxicity with more sensitive parameters to confirm our data.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-11-07

Title: Duration of fasting before taking lapatinib is associated with skin toxicity in neoadjuvant treatment of HER2 positive breast cancer: A cohort study from JBCRG-16/Neo-LaTH

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Body: Background: In neoadjuvant dual HER2 blockade, over 30% of patients fail to complete treatment as planned because of lapatinib-induced diarrhea, rash, and hepatotoxicity. Lapatinib bioavailability, which affects both efficacy and toxicity, is influenced by prandial conditions.

Methods: To investigate the association between lapatinib dosage timing and toxicity, we reviewed the medical records of patients who were enrolled in the JBCRG-16/Neo-LaTH randomized phase II multicenter trial evaluating the efficacy and safety of neoadjuvant 1000 mg/day lapatinib (La) and trastuzumab (T) therapy for 6 or 12 weeks followed by 750 mg/day La, T and weekly paclitaxel for 12 weeks in Japanese patients with primary HER2 positive breast cancer. Lapatinib dosage timing was divided into three groups: after overnight fasting, between meals, and at bedtime. We also measured serum lapatinib concentrations at steady state and dosage timing on the day prior to pharmacokinetic blood sampling. The primary endpoint was to investigate the association between lapatinib dosage timing and frequency of \geq grade 2 diarrhea. The secondary endpoint was to assess the association between dosage timing and other toxicities, pharmacokinetics, efficacy, and treatment discontinuation. Statistical analyses performed included one-way ANOVA, Welch's test and logistic regression.

Results: Out of 213 patients enrolled in JBCRG-16/Neo LaTH, we obtained dosage timing data from 143 (67%) patients: 16 (11%) after overnight fasting, 53 (37%) between meals, and 74 (52%) at bedtime. Serum lapatinib concentrations were obtained in 34/143 (24%) of patients. Dosage timing was not associated with \geq grade 2 diarrhea (8/16 (50%) after overnight fasting, 18/53 (34%) between meals, and 26/74 (35%) at bedtime; $p = 0.48$). However, multivariate analysis revealed that the after overnight fasting group is less likely to develop acne-like rash during La + T treatment regardless of age, BMI, or treatment.

Multivariate logistic regression analysis of factors predicting rash during La + T treatment

Factor		Adjusted odds ratio	95% confidence interval	p value
Age (years)	≥ 55	Reference		
	< 55	2.67	1.18-6.31	0.018*
BMI (kg/m ²)	≥ 23	Reference		
	< 23	1.04	0.45-2.39	0.933
La + T duration	6 weeks	Reference		
	12 weeks	3.62	1.49-9.77	0.004*
Concurrent endocrine treatment	Yes	Reference		
	No	2.17	0.94-5.15	0.068
Dosage timing	After overnight fasting	Reference		
	Others	3.68	1.16-11.9	0.027*

BMI cut off is based on Asian criteria for overweight status. La: Lapatinib, T: Trastuzumab, *statistically significant

In addition, serum lapatinib trough concentration and its variability were significantly reduced in the after overnight fasting group (mean \pm standard deviation (SD) = 0.35 ± 0.15 $\mu\text{g/ml}$, coefficient of variation (CV) = 42.7%) as compared to the others (mean \pm SD = 0.77 ± 0.44 $\mu\text{g/ml}$, CV = 57.8%) ($p < 0.01$). The chance of pCR was not associated with dosage timing (8/16 (50%) after overnight fasting, 24/53, (45%) between meals, and 38/74 (51%) at bedtime; $p = 0.79$).

Conclusions: These data suggest that overnight fasting stabilizes the bioavailability of lapatinib, which may aid in managing lapatinib-induced rash without diminishing its therapeutic efficacy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-11-08

Title: Stomatitis in patients treated with first-line everolimus (EVE) plus letrozole (LET): Results from BOLERO-4 trial

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Body: Background

Stomatitis is the most frequent adverse event reported in trials of mTOR-inhibitors, including EVE. In the pivotal phase 3 BOLERO-2 study, stomatitis incidence in the EVE + exemestane (EXE) arm was 59%. The BOLERO-4 study (NCT01698918) evaluated the efficacy and safety of first-line EVE + LET in postmenopausal pts with HR+, HER2- metastatic or locally advanced breast cancer (ABC). BOLERO-4 also assessed the effectiveness of an alcohol-free dexamethasone (0.5 mg/ 5ml; DEX) oral rinse for treating stomatitis in a subset of pts (USA).

Methods

Postmenopausal pts with HR+, HER2- ABC previously untreated for advanced disease received EVE (10 mg/day) + LET (2.5 mg/day). At disease progression, pts were offered EVE (10 mg/day) + EXE (25 mg/day). Pts who had at least one episode of stomatitis received oral stomatitis daily questionnaire (OSDQ), which is a 6 question pt-reported outcome (PRO) survey (Stiff et al, JCO. 2006). A subset of these pts (USA) was randomized (1:1) to receive DEX or standard of care (SOC). The primary objective of investigator-assessed progression-free survival in the first-line setting for ABC was presented previously. A secondary objective was to evaluate the effectiveness of the DEX oral rinse in reducing the severity and duration of stomatitis, using OSDQ data.

Results

Of the total 202 pts enrolled in this study, 52 pts were enrolled in USA, of which, 24 (46.2%) were randomized to receive DEX (n=11) or SOC (n=13), upon confirmation of stomatitis. The median duration of first stomatitis episode was longer per OSDQ (DEX, not estimable vs SOC, 13.7 wk) compared with physician-reported duration (DEX, 1.6 wk vs SOC, 1.9 wk). PRO OSDQ results were similar in both arms.

Among the 202 pts enrolled, 89 (44.1%) filled the OSDQ at their first stomatitis episode. The median time from treatment initiation to first stomatitis episode was 1.7 wk; median duration of stomatitis was 13.7 wk (OSDQ) vs 2.1 wk (physician reported). The majority of pts experiencing stomatitis had moderate/little/no soreness, moderate/low/no pain, and stomatitis had low/no effect on daily activities (Table 1).

Table 1. OSDQ Key Results (N=87)

Questions (Score)	First Day of Stomatitis Episode, n (%)	End of First Stomatitis Episode, n (%)
Overall health		
Poor (0-4)	20 (23.0)	23 (26.4)
Moderate (5-7)	40 (46.0)	32 (36.8)
Perfect (8-10)	27 (31.0)	32 (36.8)
Mouth and throat soreness		
No/a little/moderate (0-2)	64 (73.6)	84 (96.6)
A lot or extreme (3-4)	23 (26.4)	3 (3.4)

Mouth pain severity		
No/low/moderate (0-4)	51 (58.6)	73 (83.9)
Severe (5-7)	24 (27.6)	10 (11.5)
Unbearable (8-10)	12 (13.8)	4 (4.6)
Effect on daily activities		
No/low (0-4)	70 (80.5)	78 (89.7)
Moderate (5-7)	11 (12.6)	4 (4.6)
High (8-10)	6 (6.9)	5 (5.7)

Conclusions

Overall, patient-reported median duration of stomatitis was longer than that reported by physicians, most likely due to differences in perceptions and the challenges in collecting and cleaning PRO data. Overall good health score was maintained in the majority of pts experiencing stomatitis and stomatitis had low/no effect on daily activities. However, these results, especially in the randomized subset need to be interpreted with caution owing to the small sample size, missing data and lack of commercially available DEX in most countries.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-11-09

Title: A p53-based strategy for protecting normal breast tissue from chemotherapy-induced damage in breast conserving therapy

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Body: Introduction: Currently, the global rate of breast conserving therapy (BCT) increased steadily. Most patients with BCT are treated with chemotherapy. However, most chemotherapeutic drugs that kill cancer cells also cause undesirable injuries to normal breast tissues. Normal breast tissue damage will further cause destruction of female secondary sex characteristics, and ultimately affect the quality of patients' lives. Therefore, it is urgent to protect the normal breast tissues for BCT patients during chemotherapy. More and more evidence shows that chemotherapy-induced normal tissue damage is mainly caused by the activation of p53 pathway, which is separate from the tumor suppressor pathway of p53. Previous studies found that use of low-dose arsenic (LDA) could temporarily and reversibly suppresses p53 activation. There are recent studies showing LDA selectively protect bone marrow and gastrointestinal tract during cancer treatment. Therefore, we hypothesize that use of LDA to temporary inhibit p53 activity will be a new strategy to protect the breast normal tissues for breast conserving patients.

Methods: Human breast epithelial cell line, MCF-10A, and three breast cancer cell lines MCF-7 (estrogen receptor and E6 expressed), MDA-MB-231 (triple negative and p53 mutated) and BT-474 (HER2 overexpressed and p53 mutated), were tested in this study. All cells were pretreated with either PBS or 100nM sodium arsenite for 12 hours, followed by 375 μ M 5-fluorouracil (5-FU) or DMSO for 24, 48 and 72 hours. Cellular viability was determined by MTT assay and cell morphology was recorded under a light microscope.

Results: Morphology changes after 5-FU treatment include: the cell density decreased, the cells became rounded in shape, the cell membrane atrophied, the cell nuclei underwent pyknosis, and the cells formed a globule with nuclear and cytoplasmic fragments surrounded by the cell membrane. LDA pretreated-MCF10A cells showed significant reduced growth inhibition by 5-FU at all detected time points as demonstrated by MTT assay

Table 1. Cell growth evaluated by MTT assay.

Time after 5-FU treatment(h)	Cell line	Control (%)	LDA (%)	5-FU (%)	LDA+5-FU (%)
24	MCF-10A	100	99.30	51.38	78.28
	MCF-7	100	97.32	68.51	65.64
	MDA-MB-231	100	94.96	69.56	69.13
	BT-474	100	98.79	71.50	71.59
48	MCF-10A	100	97.37	29.13	47.74
	MCF-7	100	91.76	49.91	48.95
	MDA-MB-231	100	93.76	9.04	8.81
	BT-474	100	92.04	60.43	61.00
72	MCF-10A	100	96.34	18.34	43.64
	MCF-7	100	91.01	42.00	39.40
	MDA-MB-231	100	93.59	6.44	5.39
	BT-474	100	91.46	35.76	36.95

and morphology observation. Interestingly, LDA treatment had negligible effect on survival in breast cancer cells.

Conclusion: Temporary LDA pretreatment selectively protected normal tissue cells, but not tumor cells from toxicity of 5-FU chemotherapy. Our findings indicated that LDA pretreatment is a potential strategy to protect normal breast tissue during chemotherapy for BCT patients.

RL and HS contributed equally to this work.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-11-10

Title: Effect of supplementation with eicosapentaenoic and docosahexaenoic omega-3 polyunsaturated fatty acid on the chemotoxicity in Mexican patients with locally advanced breast cancer (LABC) treated with neoadjuvant chemotherapy (NeoCT)

DelaRosa-Oliva F, Ruiz-Garcia E, Gomez E, Arce-Salinas C, Lara-Medina F, Alvarado-Miranda A, Matus-Santos J, Flores-Díaz D, Deneken-Hernandez Z, Bargalló- Rocha J, Cabrera P, Guadarrama-Orozco J, Ruiz-Calzada H, Meneses-García A and Astudillo-de la Vega H. Instituto Nacional de Cancerología, Mexico, DF, Mexico; Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico, DF, Mexico; Hospital General ISSSTE, ZACATECAS, Zacatecas, Mexico and Hospital de Oncología, Centro Medico Nacional Siglo XXI, IMSS, Mexico, DF, Mexico.

Body: The aim of this study was to determine the benefits of supplementation with eicosapentaenoic (EPA) and docosahexaenoic (DHA) polyunsaturated fatty acids (PUFA) in terms of chemotoxicity and inflammatory status, to Mexican women with locally advanced breast cancer (LABC), receiving a standard neoadjuvant chemotherapy (NeoCT AC-taxol regimen). We performed a randomized double-blind, placebo-controlled, clinical trial, in which we studied 44 LABC patients (stages IIA to IIIA) who took 2.4g/d (1.6 g EPA and 0.8 g DHA, ratio 2:1) (N=22) or placebo (N=22) during NeoCT with doxorubicin/ cyclophosphamide followed paclitaxel weekly. Edmonton Scale and Common Terminology Criteria for Adverse Events (CTCAE) v4. assessment were performed before each CT, and also blood total leukocytes, and percentage lymphocytes and monocytes by standard lab methods. Serum inflammatory proteins and adipokines, were quantified by multiplexed analysis using a luminometer (MAGPIX) at basal time (0) and after cessation (+6 months) of AC and paclitaxel regimen.

RESULTS: There were no differences in age between the supplemented and the placebo groups (51.6y vs. 49.1y, $p=0.4$), neither in body mass index (BMI, 28.7 kg/m² and 28.9 kg/m², $p=0.8$). After 6 months of supplementation, patients who were supplemented with PUFAs had significant changes (time 0 vs 6, respectively) in total leukocytes (from 6.3 to 5.1 x10³/mL, $p=0.002$) lymphocytes (from 33.5 to 25.2%, $p=0.002$), leptin (from 60.2 to 36.1 pg/mL, $p=0.04$) and adiponectin (from 36.8 to 43.3 mg/mL, $p=0.05$). On the other hand, placebo group had significant changes in the number of leukocytes (from 6.8 to 10.0 x10³/mL, $p=0.04$), monocytes (from 6.7 to 10.1%, $p=0.03$) and an increase in leptin (from 22.7 to 46.3 pg/mL, $p=0.04$).

CONCLUSIONS: Supplementation with 2.4g/d of EPA and DHA (ratio 2:1) during 6 months in Mexican LABC patients receiving NeoCT resulted in a less inflammatory status when compared to patients who received none. Our results clearly suggest that this type of supplementation may be beneficial to these type of patients. Currently, we are analyzing if treatment with omega-3 PUFAs are associated with lower incidence of side effects due to chemotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-12-01

Title: Comprehensive genomic profiling of 34 cases of breast angiosarcoma

Ravi V, Madison R, Schrock AB B, Cote G, Millis S, Alvarez R, Choy E, Katz D, Chung J, Gay L, Miller VA A, Ross JS S, Stephens PJ J, Ali SM M and Schnitt S. Foundation Medicine, Inc; Massachusetts General Hospital; Dana Farber Cancer Institute; Southeastern Regional Medical Center; Beth Israel Deaconness Medical Center and Hadassah-Hebrew University Medical Center.

Body: Background: Angiosarcoma of the breast (BAS) is a rare but lethal neoplasia, either arising de novo or secondary to radiation therapy, with incidence of the latter disease increasing. We queried a database of more than 70,000 advanced cancer patients assayed with comprehensive genomic profiling (CGP) in the course of clinical care to uncover the frequency, type and associated genomic alterations (GA) in BAS and to highlight possible routes to benefit from targeted therapy.

Methods: CGP was performed for 34 BAS cases using a hybrid-capture, adaptor ligation based next generation sequencing assay of up to 315 genes to a mean coverage depth of >500X. The results were analyzed for base substitutions, short insertions and deletions, selected rearrangements, and copy number changes. RNA sequencing for 265 genes was also performed for 24 cases. Limited clinical histories from submitted pathology reports were reviewed under IRB permission.

Results: Clinical specimens from 34 BAS patients, all females, were assayed. The cases harbored 87 total GA for a mean of 2.59 per case, 25% of which were copy number amplifications. The most commonly altered genes were *MYC* (41%, 14/34), *PIK3CA* (26%, 9/34), and *KDR* (26%, 9/34). All *MYC* alterations were amplifications with a mean copy number of 39, and alterations in other *MYC* family members (*MYCN* and *MYCL1*) were not observed. *KDR* was recurrently altered as T771R (7/9) and T771K (1/9) and amplified in one case (1/9).

MYC and *KDR* alterations were mutually exclusive ($p < 0.0001$). 6/14 *MYC* amplified cases had prior histories of breast carcinoma, with 3/6 noted as being treated with radiation therapy. For the remainder of *MYC* amplified cases (8/14), no relevant clinical history was available.

Two cases harboring gene fusions were identified including *CIC-MEGF8* and *NTRK1-PEAR1*. Two rearrangements of potential functional significance including *CIC-DEDD2* and *HT-ALK* (exon1 *HT* - exon5-29 *ALK* including kinase domain) were also observed. The case harboring *HT-ALK* also had *MYC* amplification and known prior radiation therapy. Two other *MYC* amplified cases also harbored targetable kinase alterations, including *FLT4* amplification (described as targetable in Ravi et al JNCCN 2016) and *FGFR3* S249C, a known activating mutation.

Conclusions: *MYC* amplification defines over 40% (14/34) of advanced BAS cases. Of *MYC* amplified cases, 28% (4/14) harbored targetable alterations of tyrosine kinases including a potential novel *ALK* fusion. *FLT4* amplification only co-occurred with *MYC* amplification, but this result was not statistically significant in this small series. *KDR* and *MYC* alteration were mutually exclusive, and 45% of non-*MYC* altered cases (9/20) harbored *KDR* alterations, which were predominantly mutations of T771. Further clinico-pathologic correlation, particularly history of radiation therapy, will be explored in this series, as well defining BAS that harbor neither *MYC* nor *KDR* alterations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-12-02

Title: Cochrane review of capecitabine for hormone receptor positive versus hormone receptor negative breast cancer

Redfern A, Lau P, White A, Hoon S, Bulsara M and Long A. University of Western Australia, Perth, Western Australia, Australia; Peter MacCallum Cancer Institute, Melbourne, Victoria, Australia; Saint John of God Hospital - Subiaco, Perth, Western Australia, Australia; Westmead Hospital, Sydney, New South Wales, Australia; Universtiy of Notre Dane, Fremantle, Western Australia, Australia and Hollywood Hospital, Perth, Western Australia, Australia.

Body: Capecitabine is an oral fluoropyrimidine prodrug which may have differential efficacy in hormone receptor (HR) positive and HR negative breast cancer. We conducted a systematic review of randomised control trials (RCT) comparing chemotherapy regimens with or without capecitabine in HR positive and HR negative breast cancer.

Methods
This review was conducted by standard Cochrane review procedures. Our protocol has been published previously. We conducted a comprehensive literature search including MEDLINE, EMBASE, CENTRAL databases, clinical trial registries and major oncology conferences for capecitabine RCTs. 1913 references were identified with 209 suitable for detailed review. 62 RCTs (40 metastatic, 13 adjuvant, 9 neoadjuvant) comparing chemotherapy regimens with or without capecitabine were identified. 21 studies (10 metastatic, 7 adjuvant, 4 neoadjuvant) reporting outcome data by HR status proceeded to full data extraction. 2 studies reported comprehensive outcome data by HR status, the remainder reporting by hazard or odds ratios only.

Results
In metastatic trials, capecitabine significantly improved outcomes for HR positive but not HR negative disease for chemotherapy alone (SO14999 trial – odds ratio (OR) for OS 0.65 (95% CI 0.47-0.89) v 0.90 (0.65-1.24) and possibly with additional trastuzumab (CHAT trial – OR for PFS 0.61 v 0.87 (significance not reported)). Significant benefit was seen for adding capecitabine to bevacizumab after taxane completion only in HR positive disease (IMELDA trial – OR for PFS 0.33 (0.22-0.50) v 0.70 (0.39-1.25)). In contrast, adding capecitabine to a taxane with bevacizumab showed the reverse with only HR negative patients benefiting (TABEA trial – OR for PFS 1.49 (0.99-2,24) v 0.51 (0.269-0.959)).

Adjuvant trials investigating the addition of capecitabine to anthracycline-taxane containing regimens or as monotherapy in elderly patients. Preliminary analysis indicates HR negative cancers benefit from the addition of capecitabine compared to non-capecitabine containing regimens. No observed benefit in HR positive disease with the addition of capecitabine was been demonstrated.

Odds ratio for outcomes by the addition of capecitabine to adjuvant chemotherapy

Outcome	No. trials	HR Group	OR	95% CI	p value
DFS	6	All	0.92	0.83-1.02	0.11
	5	HR positive	1.05	0.91-1.22	0.50
	4	HR negative	0.72	0.58-0.88	0.002
RFS	1	All	0.88	0.71-1.09	0.07
	1	HR positive	0.99	0.77-1.27	0.94
	2	HR negative	0.63	0.46-0.87	0.005
OS	6	All	0.90	0.80-1.03	0.12
	1	HR positive	0.71	0.45-1.12	0.14
	1	HR negative	0.64	0.44-0.93	0.02

Neoadjuvant trials investigating the utility of capecitabine to anthracycline-taxane containing regimens showed no additional

benefit with respect to pCR. Meta-analysis of response rates for all patients and differentiated by HR status showed no significant difference on inclusion of capecitabine.

Conclusion

In summary, metastatic and neoadjuvant trials did not show clear differential benefit of capecitabine in HR positive versus HR negative breast cancer. HR positive tumours in the metastatic setting might be more responsive to capecitabine. Additional benefit to an anthracycline-taxane containing regimens with capecitabine in the adjuvant setting was confined to HR negative cancers. Final results of our analysis will be presented.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-12-03

Title: Prospective study of acupuncture in the rehabilitation of women undergoing surgical treatment of breast cancer in relation to the strength and quality of life

Giron PS Santolia, Haddad CA Assad, Rizzi SL Lopes, Pinheiro TL Lucia, Luz RP Pitta, Nazário AP Pinto and Facina G. UNIFESP, São Paulo, Brazil.

Body: Objective: To investigate the benefits of acupuncture in relation to the shoulder muscle strength and quality of life in women undergoing surgical treatment for breast cancer.

Methods: Women with breast cancer, aged 18 years or more, with pain level ≥ 3 in the Visual Analog Scale in shoulder girdle or upper limb three months after surgery, were included in this prospective study. The patients were randomly divided into two groups and received weekly treatment for 10 sessions. Kinesiotherapy group (G1): treated with pre-defined standard kinesiotherapy, lasting 30 minutes. Group Acupuncture + Kinesiotherapy (G2): treated with the same Kinesiotherapy group protocol followed by another 30 minutes of acupuncture using pre-defined points. Acupuncture points used were as follows: CV 3, SP 9, ST 36, KD 7, LV 3, GB 21, LI 15, SJ 14, LU 5, LI 4, ST 38 and BL 60. Patients were evaluated at baseline, after five weeks and at the end of 10 weeks. Parameters studied were: clinical profile and socio-demographic data, muscle strength and quality of life.

Results: 48 patients completed treatment, 24 in each group. Regarding shoulder muscle strength, all movements showed improvement and there was no statistical difference between groups. However, when analyzing intragroup changes, improvement of flexion and internal rotation were observed in G1 and increase of all movements were found in G2. Concerning EORTC, there was statistically significant difference between groups of pain symptom in the 5th session, with better score in patients of G2 in relation to G1. About EORTC intragroups results, G1 showed improvement in physical function, pain and insomnia; and G2 in performance roles, fatigue and pain.

Conclusion: According to this prospective study, kinesiotherapy and acupuncture had a positive effect on the shoulder muscle strength and quality of life of patients.

Keywords: 1. Breast cancer. 2. Rehabilitation. 3. Acupuncture. 4. Muscle Strength. 5. Quality of life.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-12-04

Title: Impact of institution of young women's breast cancer clinic on time to treatment and utilization of fertility, genetics and social work consultations in women under age 50 with new diagnosis of early stage breast cancer

Kruse ML L, Raska P, Abraham J, Budd GT, Montero A, Grobmyer S and Moore H. Cleveland Clinic, Cleveland, OH.

Body: Background: Genetic counseling and fertility resources are often underutilized in young women with early stage breast cancer (ESBC) due, in part, to concerns about treatment delays. At our institution, women newly diagnosed with ESBC typically see a breast surgeon, medical oncologist and radiation oncologist in a multidisciplinary clinic with additional cancer related subspecialist referrals occurring at those providers' discretion. We hypothesized that time to treatment (TTT) and utilization of fertility, genetics and social work consultations would improve after implementing a Young Women's Breast Cancer Clinic. As of January 1, 2015, all patients under age 50 seen at Cleveland Clinic for new diagnosis of ESBC were automatically offered scheduling of appointments with medical genetics, reproductive endocrinology and social work in addition to the usual multidisciplinary team.

Methods: Women under age 50 diagnosed with ESBC seen at Cleveland Clinic from 1/2014-12/2015 were identified using our tumor registry. Demographics, tumor pathology, clinical and treatment histories were obtained through medical chart review as per IRB approved protocol. Time from initial visit in our system to date of treatment initiation was calculated for all patients and compared between the 2014 (pre-intervention) and 2015 (post-intervention) cohorts as was time from diagnosis (biopsy date) to treatment initiation. Completed reproductive endocrinology, genetic counseling and social work consultations were documented. Welch two sample t-test was used to compare time to treatment between groups. Chi squared test was used to compare frequency of subspecialty consultations between groups.

Results: 207 young women with ESBC were identified over the 2 year period, 99 in 2014 and 108 in 2015. Median age was 45 in 2014 and 44 in 2015. Most were diagnosed outside of our hospital system, 58% in 2014 and 76% in 2015. The most common initial treatment was surgery with reconstruction (S+R) (54% and 50% for 2014 and 2015 respectively) followed by chemotherapy (23% and 27%) then surgery without reconstruction (S) (20% and 24%). Median TTT from first encounter was 30 days in 2014 and 28 days in 2015 ($p=0.33$) and was 36 days versus 33.5 days ($p=0.23$) when calculated from biopsy date. TTT in the S and S+R groups was 37 vs 28 days ($p=0.84$) and 36.5 vs 32 days, ($p=0.21$), respectively. Genetics, reproductive endocrinology and social work consults in 2014 vs 2015 were documented as 89% vs 94%, 4% vs 9% and 58 vs% 55% ($p=0.22, 0.32, 0.77$). For patients under age 40, 27% in 2014 and 30% in 2015 completed reproductive endocrinology consultations.

Conclusions: Offering upfront scheduling of breast cancer related subspecialty appointments for young women with newly diagnosed ESBC did not significantly improve overall TTT. There was a trend towards improved TTT in those receiving surgery with or without reconstruction as first treatment and no suggestion of delay in TTT. A modest numeric increase in completed genetic counseling and reproductive endocrinology consultations was not statistically significant, but may have been clinically meaningful for affected individuals.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-12-05

Title: Predictive value of serum anti-Mullerian hormone (AMH) in the outcome of emergency fertility preservation treatments indicated by breast cancer

Rodriguez-Wallberg KA A and Wikander I. Karolinska Institutet, Stockholm, Sweden and Section of Reproductive Medicine, Karolinska University Hospital, Stockholm, Sweden.

Body: Purpose:

Fertility preservation procedures aim at offering young cancer patients an opportunity to build their future families and have biologically-related children. Optimization of fertility preservation options is needed to maximize the chances to success, as the procedures are usually performed as an emergency and they cannot be repeated in most of the patients.

We aimed at investigate the predictive value of serum concentrations of Anti-Mullerian Hormone (AMH) in the outcome of emergency controlled hormonal stimulation (COS) treatments aimed at fertility preservation in women with breast cancer.

Patients and methods:

Prospective cohort study. Patients with breast cancer of reproductive age were included in the study at time of counseling for emergency fertility preservation indicated by planned chemotherapy between January 2012 and May 2016. All fertility preservation treatments were performed at the Reproductive Medicine Clinic of Karolinska University Hospital. The study cohort included 124 women with breast cancer that underwent COS cycles using an antagonist protocol either with or without letrozole supplementation, depending on their tumor estrogen receptor status. Blood draws for estimation of serum AMH concentrations were sampled previously to breast cancer treatment initiation and immediately before performance of fertility preservation.

Main Outcome measures:

The main outcome was the association of baseline serum AMH concentration, crude and age-adjusted, with the number of obtained and cryopreserved oocytes or embryos during the subsequent fertility preservation treatment.

Results

Our preliminary analyses indicate that AMH levels estimated previously to a fertility preservation treatment in women with breast cancer might be a reliable predictor of the outcome of fertility preservation in some age subgroups. The association of AMH levels with fertility preservation outcomes did lack significance in the women that were youngest at time of breast cancer diagnosis, indicating that novel biomarkers are needed in this particular patient group to optimize their fertility preservation treatments. Additionally, women that were on oral contraceptives previously to their breast cancer diagnosis presented with unusually low AMH levels for their age, which also did not correspond to their response to fertility preservation. A logistic regression analysis of the outcome including the chosen variables will follow.

Conclusion

The predictive value of AMH concentration before COS in emergency fertility preservation for women with breast cancer is still debatable. Measurements of AMH concentrations to evaluate ovarian reserve previously to fertility preservation might not correlate with the outcome in all patients with breast cancer and novel biomarkers should be investigated.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-13-01

Title: Differential protein expression in primary breast cancer tumors spreading to liver or elsewhere

Goldberg H, Fell R and Apel-Sarid L. The Galilee Medical Center, Nahariya, Israel.

Body: Breast cancer (BC) outcome is determined mainly by its ability to spread to distant sites, since this is the lethal phase of the disease. Metastasizing cells have to acquire various molecular aberrations in order to go through the metastatic cascade and gain organ tropism. These are probably reflected at the primary tumor. This study was aimed to identify a set of abnormally expressed proteins in primary human BC tumors, which spread preferentially to the liver or elsewhere. **Methods:** We collected archival paraffin embedded primary BC samples from 48 patients who were divided into 3 groups: 10 who developed liver metastases (A), 20 with metastases to other sites (B) and 18 with no BC recurrence (C). Tissue microarray (TMA) were constructed, sliced and subjected to immuno-histochemical staining. A pre-defined panel of 11 proteins was selected, based on published data that were available at the time of study initiation. Level of expression (coded by IRS scoring) and the intra/extracellular location of each protein was determined by an expert pathologist and compared between the three groups. The panel included: cell-cell interaction proteins (*CDH1*, *BIGH3*, *MMP14*, *CD44s*, *Galectin-3*), transcription factors (*FRA-1*, *c-Jun*, *GATA-3*, *TP53*), an inflammatory chemokine (*CCL5*), cell signaling protein (*Wnt-5a*). **Results:** Metastatic tropism to the liver was studied by comparing results between A and B. When similar, A and B were combined and compared with C group. *CDH1* protein expression was significantly reduced in the cytoplasm in groups A and B in comparison to C ($p=0.004$) and over-expressed in the membrane ($p<0.001$) in both. Hence, cytoplasmic *CDH1* was higher in group A than B ($p=0.03$). In group A only, a trend of *BIGH3* over-expression was noted, though it did not reach significance. No further differences were found between A versus B. *MMP14* was over-expressed both in the membrane and cytoplasm ($p=0.001$) of A and B, in comparison with C. *CD44s* was over-expressed in the membrane in A&B versus C ($p<0.001$). *Galectin-3* was over-expressed in the cytoplasm ($p=0.005$) and down regulated in the nucleus ($p=0.015$) in both metastatic groups. *CCL5* (*RANTES*) level was significantly reduced in the cytoplasm in A & B ($p<0.001$) while significantly over-expressed in the nuclei ($p=0.005$). *c-Jun* (part of AP1 complex) was over-expressed in both A and B ($p=0.021$). On the other hand, levels of *FRA-1*, localized in both cytoplasm and nucleus in C, were reduced in both compartments in A & B ($p=0.029$). No difference was noted between the 3 groups in level and localization of *TP53*, *GATA-3* and *Wnt-5a*. **Conclusions:** Assessment of the expression of proteins in tumor cells should include protein localization besides its level of expression. Both *CDH1* and *BIGH3* seem to be over-expressed in tumors that spread preferentially to the liver. No significant changes in pattern of expression of the other proteins studied could be correlated with metastatic propensity to the liver. Compartmental translocation of various proteins is correlated with the acquisition of metastatic potential, such as *CDH1* and *CCL5* which presented a shift from nucleus to cytoplasm, and *Galectin-3* and *CCL5*, where translocation from cytoplasm to membrane were observed in both metastatic groups.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-13-02

Title: Risk Factors for high risk breast density patterns in Hispanic/Latinas living in the Northeast, US

Jones BA A, Claye E, Philpotts L, Hooley R, Silber A and Epstein L. Yale School of Medicine, New Haven, CT.

Body: Introduction: Breast densities are the non-fat (epithelial and stromal) breast tissue observable on screening mammograms. Known to be associated with a 4 to 6 fold increase in breast cancer risk, dense breast patterns complicate the reading of screening mammograms resulting in lowered sensitivity. Little is currently known about the prevalence and/or predictors of breast density in Hispanic/Latino women living in the Northeast, US. **Objective:** The goal of this prospective study is to identify risk factors for high risk breast density patterns among a large cohort of Hispanic/Latino women with ancestral ties primarily to the Eastern Caribbean (Puerto Rico and the Dominican Republic) as well as Central and South America. Because the hormonal milieu is somewhat different in Hispanic/Latinas compared with White women, and likely also differs from that of Hispanic/Latinas living in other parts of the US, we explored the role of reproductive and physiological factors in high risk breast density patterns.

Methods: We analyzed breast density predictors in an established cohort of 1,600 Hispanic/Latino women recruited from primary care clinics in 4 cities with the largest enclaves of Hispanic/Latinos in Connecticut. Hour-long telephone interviews provided baseline data on biological, medical care, and sociodemographic factors. We retrieved radiology records on 1570 (98.7%) consenting women. For this analysis, we report predictors of breast density among the 1,040 (65.4%) women who received at least one screening mammogram during 2 to 4 years of follow-up. Breast density classification was based on radiologist assigned BI-RADS classification. Associations between predictors and high risk breast density patterns were examined using chi square tests. Multivariate analyses were conducted using logistic regression; odds ratios (OR) and 95% confidence intervals (CI) are reported.

Results: Of the 1,040 women who received at least one screening mammogram during the follow-up period, 280 (27%) women were identified as having high risk dense breasts (extremely or heterogeneously dense, i.e., greater than 50% of the breast composed of fibroglandular densities), while 760 women (73%) were classified as having nondense breasts (fatty or scattered fibroglandular densities), suggesting a lower prevalence of high risk breast density patterns in this population. In multivariate analysis, breast density predictors were similar to those reported for the general population (mostly White) women, and are similar to known risk factors for breast cancer. However, we found that Hispanic/Latinas with diabetes to be at significantly reduced odds of high risk breast density patterns. There was also evidence that the relationship between age at menarche and density was modified by BMI, with early age at menarche a risk factor among normal and underweight women only.

Conclusion: Our findings suggest that Hispanic/Latino women may differ in breast density prevalence relative to the general population. However, we observed a protective effect of diabetes and a potential interaction between age at menarche and BMI. This investigation enhances our understanding of breast density in a subset of the Hispanic/Latino population and provides the basis for further research.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-13-03

Title: Breast cancer education, counseling, and support for Jewish women: Preliminary results of a CDC program evaluation

Fleischmann A, Silber E, Dubitsky S, Stahl S, Kravitz S, Johnson A, Murphy S and Tercyak KP P. Sharsheret, Teaneck, NJ and Georgetown Lombardi Comprehensive Cancer Center, Washington, DC.

Body: Background: Information and social support for women at risk for and surviving breast cancer are essential components of comprehensive clinical and public health practice in breast cancer prevention, control, and treatment. Jewish women in the US are a population of special concern due to their increased hereditary breast cancer risk for carrying BRCA1/2 gene mutations, and the dearth of culturally-relevant resources for breast cancer education and counseling. As part of a CDC initiative, Sharsheret (a non-profit breast cancer support and advocacy organization) is scaling-up its programs and services for women of all Jewish backgrounds to better meet these needs, guided by program evaluation data on client engagement practices. This abstract describes these practices and survey results.

Methods: Evaluation surveys were individually-administered via e-mail using a secure online platform. Survey invitations were sent to participants ~30 days following their utilization of Sharsheret program services. Evaluation domains included program engagement and satisfaction, health-related quality of life, and resource needs.

Results: The survey response rate was >65% (N=111), and a majority of respondents reported that they were Jewish (75%), married (76%), either or 35-45 years old (35%) or 46-64 years old (48%), and from the northeast region of the US (56%). Most respondents (88%) reported a previous breast or ovarian cancer diagnosis, and 7% indicated they carried a genetic mutation known to increase breast cancer risk. Based on the CDC's 'Health Days' measures, respondents reported a higher number unhealthy days ($M=12$ days per month) relative to general population averages for adult women ($M=6.9$ days). Respondents rated their experience with the organization highly ($M=4.7$ out of 5) for overall satisfaction with the quality of services utilized. There was also strong agreement among respondents ($M=4.6$ out of 5) about the availability and utility of patient navigation services, and the survivorship program garnered the highest level of engagement (71%) as it was utilized most often by respondents for its healthy living and nutrition resources.

Conclusion: Nonprofit organizations devoted to cancer support and advocacy fill important roles in educating and counseling women about breast cancer prevention, control, and treatment. In an era of precision medicine and discussion of population-based screening for hereditary breast cancer risk, these organizations must scale-up their information and support services. This is necessary to be available for a greater number of women, and can be expected to perform even more important and essential roles, especially for Jewish women at risk for and surviving breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-14-01

Title: Metastatic trial search: Advocacy groups collaborate to engage metastatic breast cancer patients in clinical trials

Cohen EJ J, Colen SL L, Dahlke DV V, Esser M, Flowers L, Guglielmino JE E, Jenkins M, Knackmuhs G, Lusen R, Mertz SA A and Esserman LJ J. UCSF, San Francisco, CA; TX A&M Health Science Center School of Public Health, College Station, TX; Young Survival Coalition, New York City, NY; Triple Step Toward the Cure, Culver City, CA; Living Beyond Breast Cancer, Bala Cynwyd, PA; Breastcancer.org, Ardmore, PA and Metastatic Breast Cancer Network, New York City, NY.

Body: Background: Less than 5% of adult cancer patients participate in trials, delaying the arrival of new therapies to the clinic. This is concerning for metastatic breast cancer patients, for whom there is no curative treatment. To facilitate metastatic patients' access to trials, BreastCancerTrials.org (BCT) partnered with five breast cancer advocacy groups to design and develop Metastatic Trial Search (MTS), a trial matching service based on BCT technology and embedded on their websites.

Approach: BCT's partners include Breastcancer.org, Living Beyond Breast Cancer, Metastatic Breast Cancer Network, Triple Step Toward the Cure, and Young Survival Coalition. MTS was seamlessly integrated on each partner's website. To find trials, users submit data about their gender, age, location, menopausal status, cancer subtype, and sites with evidence of disease and in return receive a list of matching trials in BCT's patient-friendly format. MTS launched October 1, 2015.

Results: Our evaluation included analysis of web traffic, an online user survey, two user focus groups, and partner interviews. MTS traffic between Oct. 2015 and May 2016 resulted in over 10,000 page views; peaks of activity correlated with social media posts. The user survey, conducted between Oct. 2015 and Jan. 2016, had 102 participants; 88% learned about MTS from our advocacy partners, mostly through email (52%) or social media (21%). Most participants (60%) cited learning about new breast cancer research as an important benefit of MTS. Sixty-three percent of users were looking for treatment trials, 16% contacted a research site and 12% spoke to their doctors about trials that they found on MTS. Among all respondents, 7% enrolled in a trial and 29% were still considering enrollment. Sixty percent of MTS users were satisfied with their experience, 33% were neutral, and 6% were dissatisfied; 65% would recommend MTS to a friend. To improve MTS, users ranked adding filters to narrow search results as the most pressing need. Providing a trial alert service was ranked second. Over 80% agreed with the statement: "MTS met an unmet need." The focus groups were made up of 14 survey respondents who volunteered to participate. Discussants spoke highly about MTS' ease-of-use and its advocacy group endorsement. They also appreciated how MTS helped them learn about different ways to participate in research and provided information to share with their oncologists. According to many participants, one of the most important benefits of MTS was that it helped them "be prepared" if they needed to change treatment. Our advocacy partners were very satisfied with their involvement and all stated that "collaboration and sharing" were the most important outcomes. In addition, they viewed the consistency of seeing MTS on each of their sites as a benefit for patients looking to advocacy groups for trusted information.

Conclusion: Our experience with MTS shows that advocacy groups working together can create an important channel for engaging patients in learning about metastatic breast cancer trials. Next steps will be to expand our advocacy network, make it easier for users to narrow search results, and offer ways for patients to learn about newly opened clinical trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-14-02

Title: OncoGambit: A revolutionary computer -assisted oncology treatment decision

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Body: Background: Over 14 million new cases of cancer (1.5 million breast cancer) will be diagnosed worldwide in 2016. It is recommended that cancer treatment should be started as soon as possible, as worse survival has been associated more than 90 day-delay in treatment (1). In the era of digital health, cancer patients resort to accessing the internet for treatment information. However, 41.3% of these patients reported being frustrated with the time consuming effort during this process. (2). Since compliance with breast cancer treatment guidelines showed 60-80% variant among treating physicians, it is imperative for patients to seek evidence-based treatment confirmations or second opinions. (3) OncoGambit was designed by experienced oncologists to provide patients with instant information regarding their treatment options based on current, up-to-date, and evidence-based treatments recommended by national guidelines (ASCO, ASTRO, NCCN, ESMO).

Method: The software was designed to provide patients with an algorithmic personalized treatment recommendations for their individual cancer, based on the patient's tumor stage and tumor molecular bio-markers. For example, the treatment for breast cancer is based on patient's age, menopausal status, cancer stage, ER, PR, and HER2neu status. In addition, much needed information such as follow up care plan and survivor care recommendation were also included. Education regarding the patient's cancer type and how its treatment derived was provided prior to the patients' inputting their information. The first version of the software was piloted and promoted on social media (Facebook and Twitter) between 10/2015 and 12/2016.

Results : During this testing period, 3792 users accessed the site, with 4870 observation sessions, as some patients reviewed more than one cancer type. Users were from 69 countries, with 69% of them being from the US. The most common cancers searched were breast cancer (30.4%), lung cancer (20.3%), melanoma (13.8%), prostate cancer (7.6%). In addition, the cancer genetics information was accessed by 12.7%. Of these early users, 8.5% proceeded to request the complete cancer treatment report.

Conclusion: The preliminary data from the early release testing for OncoGambit version 1.0 demonstrated the need for an instant access cancer treatment application. Treatment confirmation is becoming a high priority for cancer patients. OncoGambit is the first revolutionary computer-aided individualized cancer treatment confirmation resource designed to confirm treatment instantly, thus reducing the time and effort required to acquiring this information from multiple websites. In addition, the information provided is based on the current cancer treatment guidelines that is updated daily, as the guidelines frequently change. OncoGambit can further facilitate patient-physician partnership by engaging and involving cancer patients to participate in their treatment plans. User feedback from the testing phase is currently used to upgrade this resource to accommodate the user's understanding of this resource and ease of use.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-14-03

Title: Novel visualization tools to search ongoing clinical trials and track trial results for metastatic breast cancer

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Body: Metastatic breast cancer (MBC) remains a terminal illness and is the cause of virtually all breast cancer deaths. The median survival for patients with MBC in the US is approximately 3 years from time of stage IV diagnosis. People living with MBC are in urgent need of new treatment options once current therapies no longer keep the disease under control. Access to new treatments is often through clinical trials. We have developed a novel way to visualize active clinical trials and track trial results.

Methods: Clinical trials were queried from Clinicaltrials.gov and the National Cancer Institute. Trials were categorized by breast cancer subtype, line of therapy, hallmark of cancer, sponsor, phase, sample size and compound. A bubble chart was created to display data triplets (x, y, z) corresponding to three continuous variables: x and y to define the position, and z, the radius of each bubble. In our example, x and y coordinates are optimized according to an algorithm that essentially balances the bubble's gravity (*i.e.*, action of moving towards the center of the plot), friction (*i.e.*, velocity decay) and charge (or repulsion/attraction force). This layout is frequently used for network visualization. In addition to x, y and z, it is possible to encode other information. **Results:** We identified 204 clinical trials actively recruiting patients with MBC. The current webpage (www.mbcalliance.org) displays clinical trial data from 204 clinical trials in six different ways. Trials actively recruiting patients will be updated quarterly. In addition to data triplets (x, y and z) we encoded other information about trials using a color code to add three additional dimensions: phase, breast cancer subtype and sponsor type. When a user hovers over a bubble, key information about the clinical trial extracted from Clinicaltrials.gov is displayed. When a user clicks on the bubble, the user is directed to the corresponding landing page at BreastCancerTrials.org to access detailed trial information. We are employing a similar coding scheme and display characteristics for completed clinical trials results. **Conclusions:** We have developed a user-friendly, web tool to quickly sort and visualize ongoing clinical trials and results for completed trials to benefit both MBC patients and research scientists.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-15-01

Title: Building an experience engine to make cancer treatment decisions using machine learning

Ramarajan N, Gupta S, Perry P, Srivastava G, Kumbla A, Miller J, Feldman N, Nair N and Badwe RA A. Navya Network, Cambridge, MA; Tata Memorial Centre, Mumbai, Maharashtra, India and Olive View University of California Los Angeles Medical Center, Los Angeles, CA.

Body: Introduction: Experts at tertiary care centers provide solutions to complex cases not addressed by high quality evidence. They intuitively retrieve patterns from years of experience to make treatment decisions. Short of personal consultations, there is no way to access this vast “experience database.” Experience Engine (XE) is a machine learning solution to structure experiential knowledge relevant for decision making, derive a similarity metric for patients who have received similar treatments, and predict treatment decisions that experts are likely to recommend.

Methods: 277 patient histories relating to 743 breast cancer tumor board decisions at two tertiary care centers were abstracted as the training set for machine learning. 161 distinct histories relating to 496 decisions for a separate expert opinion service at one of the centers was the holdout test set. Data was structured into 690 features based on a novel ontology designed specifically for breast cancer decision making.

To uncover nonlinear similarities, (for example, treatments for younger patients with multiple comorbidities and elderly patients may be similar), treatment decisions were grouped by timing and modality into 13 groups, such as primary surgery, 1st line palliative chemotherapy, etc.

Similarity metric was derived using machine learning on the training set. The target for prediction was the specific treatment decision i.e. TAC or another adjuvant regimen. The primary endpoint was percent accuracy of agreement between XE's predicted decision and experts' actual decision in the holdout test set. Multiple similarity distance metrics including Bhattacharya, Eskin, Goodall, etc., and multiclass classification algorithms such as Extreme Gradient Boosted Trees, Support Vector Machines, etc., were systematically evaluated to arrive at the algorithms that best fit each treatment group.

Results: The winning XE algorithms were 71% to 89% accurate for the various treatment groups, in predicting the actual treatment decisions recommended by the experts. The most frequent treatments recommended across all groups were standard evidence based therapies, as are often recommended by experts. For instance, when XE recommended standard adjuvant therapies for Her2- patients, it was 88% to 97% accurate. When XE recommended nonstandard therapies for the same treatment group, it was 72% to 90% accurate, related to larger number of nonstandard therapies within each treatment group and smaller samples of patients who underwent each type of nonstandard therapy. XE learned to weigh features relating to comorbidities and toxicities when recommending nonstandard therapies.

Conclusion: Machine learning on a structured database of past treatment decisions made by experts, can yield a predicted treatment decision that an expert is likely to recommend for a new patient. By including complex decisions that consider toxicities and morbidities, a rich source of knowledge can be created. Despite the limited dataset, XE learned features that experts strongly consider when making decisions. XE has the potential to analyze variations in decision making at expert practices, assess when to recommend nonstandard therapies, and serve as a training tool for new oncologists to make expert grade treatment decisions.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-15-02

Title: Attitudes towards tissue donation for rapid autopsy among community and academic oncologists

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Body: Rapid autopsy (RA) refers to the recently developed practice of obtaining research tissue within 2-6 hours following the death of a patient. Such tissue can offer insights into the genomic and proteomic evolution of metastatic disease and possible novel therapeutic targets. Although patients generally have positive attitudes towards RA, RA programs have not been widely established by oncologists at healthcare centers with these capabilities. To identify barriers to our adoption of an RA program, we conducted a 21-item web-based survey of academic and community oncologists in a single institution with regard to knowledge, attitudes and concerns about RA. Twenty-six physicians completed the study out of a possible 98 respondents, with 50% in academic practice and 50% in community practice. 46% were not aware of RA prior to the survey. None of the community physicians had experience with patient tissue donations of any kind. In completing the survey, 85% were willing to ask future patients about participating in RA. 58% of oncologists cited lack of awareness as their primary barrier to participation, while 31% reported discomfort in discussing rapid autopsy with patients or concern about the doctor-patient relationship. A minority of physicians had ethical concerns about the practice. The most popular strategies for increasing awareness of the RA program included distributing informational pamphlets to patients, using a third-party advocate or a discussion with the patient's primary oncologist or NP. 92% of those surveyed stated that being aware of patient interest would make them much more likely to recommend RA to patients. Our study is among the first to clarify barriers among oncologists, particularly community oncologists, to establishing RA programs and suggests that increased information alone with clarification of patient perceptions could provide substantial progress in developing RA programs at tertiary healthcare centers and recruiting patients to these programs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P2-16-01

Title: Relationship between overall survival and surrogate measures in patients with metastatic breast cancer treated with chemotherapy

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Body: Background: While metastatic breast cancer (MBC) is considered an incurable disease, nearly one-fifth of patients live longer than five years following diagnosis. In an effort to identify novel agents earlier, surrogate end points of overall survival (OS) such as response or progression are often employed in randomized controlled trials (RCTs). The goal of this analysis is to evaluate patterns of outcome reporting and the relationship of OS with surrogate measures in RCTs of patients with MBC.

Methods: The analysis was based on data from a systematic review of patients with MBC evaluating the clinical impact of chemotherapy intensity on survival. Reports of phase 2-3 RCTs published between 1990-2013 comparing more intense chemotherapy regimens (higher dose intensity or use of additional agents) with less intense were identified. For each RCT, clinical, treatment, demographic and outcome data were extracted. Outcomes evaluated included OS, progression free survival (PFS), and time to progression (TTP) with a focus on median survival and hazard ratios (HRs) as measures of treatment effect. Survival post progression (SPP) was calculated as the difference between median survival and median progression free time. The relations between various outcome measures were estimated utilizing weighted Pearson correlation coefficient (CORR) adjusted by Fisher's transformation. Weights were assigned proportionally to the sample size of individual RCTs.

Results: The review identified 70 eligible RCTs including 15,043 patients with MBC. Average median OS, PFS, and TTP were 19.2, 6.9, and 8.1 months reported in 96%, 60%, and 43% of studies, respectively. Progression could be determined in 66 studies, while 6 RCTs provided both outcomes. TTP was more often utilized in earlier studies (65% in 1990-2000, 35% in 2001-2008, and 33% in 2009-2013) and it was superseded by PFS in later years (20%, 70% and 81%, respectively). Only 37%, 33%, and 11% of RCTs reported HRs for OS, PFS, and TTP, respectively. HRs were more often available in recent publications (20% in 1990-2000, 22% in 2001-2008, 63% in 2009-2013 provided HR for OS). The correlation between reported HR and HR estimated by the ratio of arm-specific median survival times was high for OS (CORR=0.87, 95%CI: 0.73-0.94) and TTP (CORR=0.92, 95%CI: 0.61-0.99) and slightly lower for PFS (CORR=0.72, 95%CI: 0.44-0.87). The relationship between OS and surrogate measures (PFS, TTP) was weaker. The correlation between HR for OS and PFS was 0.49 (95%CI: 0.21-0.69) and for OS and TTP it was 0.26 (95%CI: -0.13-0.58). Survival time following progression was dependent on treatment type and was longer in less intense arms than more intense (mean SPP: 12.4 months vs. 11.4 months, P=0.0155).

Conclusions: In RCTs of patients with MBC treated with chemotherapy, when HR is not reported and if necessary statistical conditions are met, the HR approximated by ratio of median survival times may be a suitable proxy estimate. In agreement with other reports, neither PFS nor TTP are acceptable surrogate outcomes for OS in MBC, as survival following progression may be substantial. In these patients, crossover and post-trial treatments may influence the relationship between OS and surrogate measures.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD3-01

Title: Automated, low-cost palpable breast lump triage for economically-developing countries

Love SM M, Berg WA A, Podilchuk C, Hovanesian-Larsen LJ J, Dauphine C, Jairaj A, Barinov L, Hulbert W, Cen S, Eshraghi L and Mammone R. Dr. Susan Love Research Foundation, Encino, CA; University of Pittsburgh School of Medicine, Magee-Womens Hospital, Pittsburgh, PA; Clearview Diagnostics Inc., Piscataway, NJ; University of Southern California, Keck School of Medicine, Los Angeles, CA; Los Angeles Biomeical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; Keck School of Medicine, Los Angeles, CA and Rutgers University, New Brunswick, NJ.

Body: Background: According to GLOBOCAN, 1.7 million women were diagnosed with breast cancer and 445,000 died from the disease in 2012. Breast cancer is the most common cause of cancer death among women worldwide and the numbers are disproportionately high for women in developing countries. Furthermore, breast cancer is more common in young women (age 44 and under) in these countries than in industrialized countries. Shifts in lifestyle typical of industrialized societies are thought to be causing a rise in breast cancer incidence while technical advances in detection, diagnosis, and treatment are not reaching these areas of the world. In developing countries, breast cancer commonly presents in women as a palpable mass and a low-cost solution for breast cancer triage would be beneficial. Ultrasound has been shown to perform very well on palpable masses, is non-ionizing, and affordable, portable systems are available. In the ACRIN 6666 clinical study of asymptomatic women, adding screening ultrasound to mammography significantly increased breast cancer detection in women with elevated cancer risk and dense breasts, and more invasive cancers were seen by ultrasound alone than by mammography alone.

Method: We propose a novel computer-aided diagnosis (CADx) tool for breast cancer triage using a low-cost ultrasound imaging device that will automatically distinguish among (1) suspicious lesions to be sent for biopsy, (2) benign findings that need no additional follow-up and (3) probably benign findings with recommended 6-month follow-up. A validation study at USC Norris Cancer Center and Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center was performed in order to assess the performance of the triage software compared to the standard-of-care and biopsy-confirmed cancers and false positives. The Study machine was the GE Logiq e ultrasound imaging device, which is FDA-approved for small body parts. Lesions that were assessed as BI-RADS 2 by radiologists were determined to be benign while any lesion assessed as BI-RADS 4a or higher was sent for biopsy. This study cohort included women 61 years of age or younger with a palpable mass. The triage software was compared to the performance by radiologists using conventional standard-of-care for palpable masses.

Results: The original milestone for this study was to achieve a sensitivity of 85% while reducing the number of benign lesions going to biopsy by 40%. The validation study showed an actual sensitivity of 100% while reducing the number of benign lesions going to biopsy by 69%. To date, the images collected during the validation study include 152 lesions in total, 22 biopsy-proven cancers and 130 benign lesions; 63 benign lesions were categorized as BI-RADS 4a or higher by a radiologist and sent for biopsy.

Conclusions: The validation study showed that adding automated breast cancer triage software to a low-cost portable ultrasound imaging device is a realistic approach for regions of the world with limited access to highly trained radiologists and diagnostic equipment.

Funding: NIH Grant #UH2EB019889.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD3-02

Title: Intraoperative tumor detection using a ratiometric activatable fluorescent peptide: A first-in-human phase I study allows tumors to be visualized in the operating room

Unkart JT T, Chen SL L, González JE E, Harootunian A and Wallace AM M. University of California San Diego, La Jolla, CA and Avelas Biosciences, Inc, La Jolla, CA.

Body: Background:

A continuing challenge in breast cancer surgery is the difficulty with intraoperative lymph node status determination and achieving negative surgical margins. Current techniques such as frozen section, touch prep and intraoperative radiographic imaging are time consuming and vary in accuracy from institution to institution. Our study is a first-in-human study of a novel ratiometric activatable cell penetrating fluorescent peptide dye conjugate that labels breast cancer tumor tissue in vivo.

Methods:

AVB-620 is a substrate for and activated by proteases in the matrix metalloproteinase family. It has two fluorophores moieties linked by a cleavable peptide. Upon cleavage, there is an increase in fluorescence intensity and a change in the predominant wavelength of fluorescence emission. AVB-620 was given preoperatively via intravenous infusion to stage 0-III breast cancer patients. Patients were monitored for safety and AVB-620 pharmacokinetic parameters determined. After 12-20 hours, patients underwent lumpectomy or mastectomy with either sentinel lymph node biopsy (SLNB) or axillary lymph nodes dissection (ALND). Using a customized near-infrared camera system, fluorescence intensity signals at two wavelengths were measured intraoperatively both in vivo and ex vivo of primary tumors, shave margins and lymph nodes. The intensity ratio of these two wavelengths was utilized to distinguish between malignant and non-malignant tissues. Images were correlated to pathology reports.

Results:

Fifteen patients with average age of 59 years were enrolled across 5 dose cohorts without any significant adverse events. All patients underwent definitive breast surgery (12 lumpectomy, 3 mastectomy) and axillary surgery (12 – SLNB, 3- ALND). Four patients that had tumor-positive lymph nodes confirmed pathologically which correlated with increased fluorescence intensity and ratio on imaging. Primary tumor images also demonstrated fluorescence intensity and ratiometric changes that distinguished cancerous and non-cancerous tissues.

Discussion:

The purpose of this phase 1 dose escalation was to determine safety profile and adequate dosing. This study demonstrated a proof of concept of the use of a protease-activatable ratiometric fluorescent peptide dye conjugate to identify malignant tissue intraoperatively. Increased fluorescent ratio correlated and resulted in fluorescent imaging of known tumor in the operating room, with images that directly correlated with position of tumor in the excised tissue. This approach has the potential to quickly and accurately visualize the pathologic status of tumor margins and lymph node intraoperatively. Further studies to optimize dose, timing of administration, and thresholds for ratiometric imaging are underway.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD3-03

Title: Determining the prognostic role of early and end-of-neoadjuvant chemotherapy 18F-FDG PET/CT in patients with locally advanced breast cancer

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Body: Aim: To investigate the use of 18F-FDG PET/CT at diagnosis and after end-of-treatment in the prediction of response to neoadjuvant chemotherapy (NAC) and its role in the prognosis of patients with locally advanced breast cancer (LABC).

Materials and Methods: One hundred thirty-two patients underwent a baseline FDG PET/CT (PET-1) after the second course of chemotherapy (PET-2) and after the last course (PET-3).

Breast tumors were classified into molecular phenotypes and grouped into risk categories according to the biological prognostic factors obtained by immunohistochemistry.

PET/CT scans were semiquantitatively evaluated, obtaining the $\Delta\%$ SUV1-2 and SUV1-3 in primary tumor and axillary lymph nodes to establish response groups attending to EORTC criteria. Moreover, a binary assessment was obtained classifying the studies as positive or negative.

Pathological response was determined both in breast and axillary lymph node specimens.

Overall survival (OS) and disease-free survival (DFS) were obtained during the follow-up.

ROC analysis was performed to determine a cutoff value of $\Delta\%$ SUV1-2 and SUV1-3 for the prediction of response and prognosis.

Relations between molecular phenotypes, metabolic behavior, final pathological response, OS, and DFS were evaluated.

This prospective and multicenter study was approved by the local ethics committee of our institution and included 7 hospitals of our region.

Results: In binary analysis, only PET-3 was able to predict pathological response in lymph nodes. The cutoff values of $\Delta\%$ SUV1-2 and $\Delta\%$ SUV1-3 with the best sensitivity and specificity in the prediction of response in breast tumor were 62% (Se: 70% and Sp: 69%) and 84% (Se: 70% and Sp: 88%). A $\Delta\%$ SUV1-3 of 74% in breast tumor was a predictor of DFS (AUC = 0.647; P = 0.037, Se: 52% and Sp: 66%). Kaplan-Meier analysis revealed significant relations between the binary lymph node assessment of PET-3 with OS (P = 0.016, $\chi^2 = 5.78$) and DFS (P = 0.003, $\chi^2 = 9.10$).

Conclusions: Among the multiple metabolic response variables in breast tumor and lymph nodes, end-of-treatment 18F-FDG PET/CT was a significant predictor of breast and lymph node response and patient prognosis. The molecular phenotypes and pathological lymph node response rate were independent predictors of OS and DFS. This stresses the potential of the biological influence over metabolic variables in the prognosis of patients. Metabolic response variables work better in their predictive and prognostic value in high-risk tumors.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD3-04

Title: Which measure of the interim changes in breast tumoral volume at breast MRI in response to neoadjuvant chemotherapy best predicts final pathological response?

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Body: BACKGROUND: Interim changes in breast tumour volume at magnetic resonance imaging (MRI) can predict ultimate response to neoadjuvant chemotherapy (NAC), but there is little data on the best measure of volumetric change.

PURPOSE: To assess whether changes in measurements of semi-automated enhancing tumour volume (ETV) or fully automated functional tumour volume (FTV) between baseline and interim contrast-enhanced MRI are equivalent in predicting ultimate pathological response to neoadjuvant chemotherapy (NAC) for primary breast cancer, assessed using the residual cancer burden (RCB) score.

MATERIALS & METHODS: 78 patients undergoing treatment with NAC for primary breast cancer underwent contrast-enhanced MRI on a 1.5T or 3.0T MRI scanner using a dedicated bilateral breast coil before and after two or three cycles of NAC. Image analysis was performed using either semi-automated, user-defined thresholding (ITK-Snap; ETV) or fully-automated (Siemens SyngoVia BreVis; FTV) approaches. For ETV, the two-minute post-contrast subtracted volumes were analysed, with enhancing pixels thresholded to define tumour volume. FTV was measured using a manufacturer default setting of 50% enhancement threshold, relative to pre-contrast signal intensity, to define tumour volume. ETV intra-observer reproducibility was assessed by repeat analysis one month after initial analysis and a second observer also repeated the measure. Coefficient of reproducibility (CoR) and intraclass correlation coefficients (ICC) were calculated for intra- and inter-observer repeatability.

ETV and FTV percentage reduction between baseline and interim examinations was compared with final pathological response, as assessed using the residual cancer burden (RCB) score on resected cancer specimens.

Correlation of the two volumetric measures was performed using a Pearson Intra-class Correlation Coefficient (ICC) and pair-wise comparisons of ETV and FTV changes between RCB groups carried out using a Mann-Whitney U test. All statistical assessment was performed using SPSS, v21, with $p < 0.05$ considered significant.

RESULTS: There was significant correlation between ETV and FTV (ICC= 0.744, $p < 0.05$). Intra and inter observer reproducibility for ETV was excellent, with ICC 0.940 and 0.861 respectively and corresponding CoRs of 11.6% and 14.8%.

Average percentage reductions in ETV for each pathological response category were: pCR 96.4% (n=12), RCB-I 66.6% (n=10), RCB-II 62.9% (n=39) and RCB-III 27.3% (n=17). Corresponding values for FTV were 88.8%, 70.6%, 54.6% and 20.8%.

Significant differences in percentage ETV changes were found for pCR vs. RCB-I ($p < 0.008$), II ($p < 0.001$) & III ($p < 0.001$) and RCB-II vs. RCB-III ($p < 0.001$). For FTV, significant differences were measured only for pCR vs. RCB-II & III ($p < 0.001$).

CONCLUSION: changes in the semi-automated ETV measurement between baseline and interim MRI may provide more useful predictive information on final pathological response to NAC than FTV, as the changes are better able to discriminate between pCR and minimal residual disease (RCB-I). The ability to confidently predict pCR versus all other residual disease categories could facilitate planning of enhanced approaches to surgical management.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD3-05

Title: Effect of MR imaging contrast kinetic thresholds for prediction of neoadjuvant chemotherapy response in breast cancer subtypes – Results from ACRIN 6657 / I-SPY 1 trial

Li W, Arasu V, Jones EF F, Newitt DC C, Wilmes LJ J, Kornak J, Esserman LJ J and Hylton NM M. The University of California, San Francisco, San Francisco, CA.

Body: Background: Breast MRI has the potential to non-invasively measure response to neoadjuvant chemotherapy (NACT). We studied the effect of varying two analytic parameters used to define MRI-measured tumor volume in the prediction of pathologic complete response (pCR) to NACT and to determine if optimization of these parameter thresholds would improve the prediction of pCR.

Methods: Women with locally advanced breast cancer (tumor size ≥ 3 cm) were enrolled in the ACRIN 6657 / I-SPY 1 TRIAL. Each patient had up to four dynamic contrast-enhanced MRI examinations: before NACT (MR1), after one cycle of NACT (MR2), between the anthracycline-based regimen and taxane (MR3), and after NACT and prior to surgery (MR4). Breast cancer was stratified by subtypes of hormone receptor (HR), and human epidermal growth factor receptor 2 (HER2) status: HR+/HER2, HER2+, and triple negative ((TN) HR-/HER2-). MRI-measured functional tumor volume (FTV) and change in FTV (Δ FTV) were investigated as predictors of the outcome pCR. FTV is defined as the image volume with enhancement kinetics exceeding both an early percentage enhancement threshold (PEt) and a signal enhancement ratio threshold (SERt). Primary study analysis used empirically determined values. For this study PEt was varied from 30% to 200% in 10% intervals, and SERt was varied from 0.0 to 2.0 in 0.2 unit intervals. FTV was measured at each examination (FTV₁, FTV₂, FTV₃, FTV₄). Δ FTV was measured relative to the first examination (Δ FTV₂, Δ FTV₃, Δ FTV₄). For each pair of varied PEt and SERt thresholds, the absolute and relative FTVs were re-measured and analyzed for discrimination of pCR using the area under the curve (AUC) of the receiver operating characteristic curve.

Results: A total of 116 patients were included from the ACRIN 6657 / I-SPY 1 TRIAL who had complete data on all four MRI visits, HR/HER2 status, and pCR outcome. Mean age was 48 years old (range 29-69). The full cohort of 116 patients was divided into subgroups: 45 (39%) HR+/HER2-; 39 (34%) HER2+; and 30 (26%) TN. When stratified by subtypes, lower AUCs with less variation were observed in patients with HER2+ cancer than patients with HR+/HER2- and TN breast cancer. When examining prediction by visit, maximum AUCs were found at later time points in all patient cohorts. Specifically, maximum AUC was observed for the full cohort at Δ FTV₃ with AUC of 0.78 (CI: 0.69–0.87) when PEt=130% and SERt=0; for HR+/HER2- subtype at Δ FTV₃ with AUC of 0.9 (CI: 0.84–0.97) when PEt=130% and SERt=0 were the same as in the full cohort; for HER2+ subtype at FTV₃ with AUC of 0.77 (CI: 0.62–0.92) when PEt=70%/SERt=1.4; for triple negative at FTV₄ with AUC of 0.89 (CI: 0.76–1) when PEt=40%/SERt=2.0.

Conclusion: This analysis suggests that the thresholds of MRI quantitative DCE measurements may need to be adjusted by breast cancer subtype to improve the predictive performance. The PEt threshold may need to be set higher in HR+/HER2- than other subtypes, which may be due to higher background parenchymal enhancement among HR+ patients. SER threshold may need to be set at higher level for triple negative subtype. A validation is underway in I-SPY 2, with a larger patient population.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD3-06

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: PD4-01

Title: Acupuncture for chemotherapy-induced peripheral neuropathy in breast cancer, preliminary results of a pilot randomized controlled trial

Lu W, Giobbie-Hurder A, Freedman R, Yung R, Lin N, Partridge A, Shockro L, Stecker K, O'Connor KA A, Rosenthal DS S and Ligibel JA A. Dana-Farber Cancer Institute, Boston, MA.

Body: BACKGROUND: Chemotherapy-induced peripheral neuropathy (CIPN) is one of the major dose-limiting side effects in breast cancer patients, with up to 97% of patients receiving an adjuvant taxane experiencing this symptom in the months and years after breast cancer treatment. CIPN often leads to loss of physical function; difficulties in activities of daily living and decreased of quality of life (QOL). Few effective interventions have been developed to alleviate CIPN in this patient population. We conducted a pilot randomized controlled trial to assess the feasibility, safety and preliminary effect of an acupuncture intervention on CIPN in breast cancer survivors.

METHODS: Patients with stage I-III breast cancer who were experiencing CIPN after the completion of a taxane-containing adjuvant chemotherapy regimen were enrolled and randomized 1:1 to immediate participation in an acupuncture intervention or to a delayed intervention control group. Participants randomized to the acupuncture arm received 18 sessions of a standardized acupuncture protocol over 8 weeks while the control group received a lower-dose acupuncture protocol consisting of 9 acupuncture sessions over 8 weeks, after the initial 8-week control period. Measures including the Patient Neurotoxicity Questionnaire (PNQ), Functional Assessment of Cancer Therapy Neurotoxicity subscale (FACT-NTX), and the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Chemotherapy-induced Peripheral Neuropathy 20 (EORTC QLQ-CIPN20) were collected at baseline and at 8 weeks after enrollment.

RESULTS: A total of 40 patients were enrolled; 20 were randomized to the immediate acupuncture group and 20 to control. All enrolled patients were female, median age was 54, median time between enrollment and completion of chemotherapy was 14.3 months, and 72.5% of participants were White. Thirty-two patients (84%) completed at least 80% of the required sessions. No serious acupuncture-related side effects were observed. Participants randomized to the acupuncture arm experienced improvements in the PNQ sensory score ($p=0.02$), FACT-NTX summary score ($p=0.002$) and EORTC QLQ-CIPN20 score ($p=0.006$), respectively equivalent to 40%, 36% and 53% improvement in CIPN symptoms, as compared to controls.

Measurements	Time points	N	Acupuncture		Usual Care		P-value
			Mean	SD	Mean	SD	
PNQ summary sensory score (0-4)	Baseline	20	2.5	0.8	2.5	0.9	0.97
	Changes at 8 week	15	-1.0	0.9	-0.3	0.6	0.02
FACT-NTX summary score (0-44)	Baseline	20	25.0	8.4	22.1	9.4	0.40
	Changes at 8 week	15	9.0	9.2	1.2	5.4	0.002
EORTC QLQ-CIPN20 sensory score (0-100)	Baseline	20	44.9	19.9	45.0	22.3	0.93
	Changes at 8 week	15	-23.8	18.1	-5.1	6.4	0.006

CONCLUSIONS: Women with CIPN after adjuvant taxane therapy for early breast cancer experienced a significant and clinically meaningful improvement in neuropathy symptoms as a result of an 8-week acupuncture protocol. Given the prevalence of taxane-induced neuropathy in women treated for early breast cancer, acupuncture could significantly improve QOL and functional status of thousands of women treated for breast cancer every year. Larger studies are needed to confirm these findings and evaluate the impact of acupuncture on functional measures in women with CIPN.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD4-02

Title: A study to determine the optimal method of detection and threshold for lymphoedema intervention: A multi-centre prospective study

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Body: Introduction

Lymphoedema, a complication of nodal surgery in 30-40% of patients, reduces quality of life for sufferers. This prospective, multi-centre study compared multi-frequency bioimpedance spectroscopy (BIS, ImpediMed) with a validated perometer method to determine which test is more sensitive for detecting lymphoedema after axillary clearance and identify the factors predicting lymphoedema development.

Material and methods

Participants (n = 629) undergoing axillary clearance at 9 UK centres underwent pre-operative and arm volume measurements post-surgery (1, 3, 6, 9 & 12 months, then 6 monthly) by arm perometry, BIS measurements (L-Dex) and recorded self-reported symptoms via questionnaires. Follow-up was a minimum of two years from surgery. Change in arm volume was calculated using relative arm volume change (RAVC) with $\geq 10\%$ increase defined as lymphoedema. The predictors of lymphoedema development and optimal method for its detection were assessed using Cox Regression, Log Rank and Kaplan-Meier survival analyses.

Results

In total, 629 women underwent axillary surgery, with a median age of 56 (range 22 to 90) years; 80% were ER positive and received endocrine therapy, 78% received radiotherapy and 65% received chemotherapy. Lymphoedema was detected by 24 months in 124 (20%) women by perometry. Using the LDex ≥ 10 cut-off score, bioimpedance sensitivity was 71% and specificity was 89% (PPV 47%) compared to RAVC changes. Women who had an RAVC $\geq 5\%$ - $<10\%$ at six months developed lymphoedema in 44% of cases by two years, whereas those who had less than 3% RAVC developed lymphoedema in 9% of cases ($p > 0.000001$). Twenty-six per cent of ER negative patients developed lymphoedema compared to 19% ER positive cancer patients.

The type (taxane versus no taxane) and whether chemotherapy was neo-adjuvant or adjuvant did not predict lymphoedema development.

Univariate analysis revealed BMI ($p=0.003$), ER negativity ($p \leq 0.010$), absence of endocrine therapy ($p=0.034$), number of nodes involved ($p=0.001$) and an increase in RAVC $\geq 5\%$ - $<10\%$ ($p \leq 0.005$) all predicted lymphoedema development by two years. On multivariate analysis, RAVC $\geq 5\%$ - $<10\%$ after six months (HR 5.51 95% CI 3.05 – 9.94) along with number of nodes involved (HR 1.06 95% CI 1.03 – 1.09) and BMI HR 1.04 (1.04 – 1.09) were included in the model for predicting lymphoedema development at two years.

Conclusions

This is the first report; ER negative cancer is associated with an increased risk of lymphoedema after axillary node clearance. Arm measurements should be taken from baseline in all patients undergoing axillary surgery and increases greater than 3% should lead to further surveillance to prevent lymphoedema development. Perometer measurement is the optimal technique for measuring and predicting the development of lymphoedema.

A threshold RAVC of $\geq 5\%$ - $<10\%$ after six months predicts lymphoedema in 44% of patients by two years.

(Funded by NIHR Programme Grant).

2016 San Antonio Breast Cancer Symposium

Publication Number: PD4-03

Title: Chemotherapy-related risk factors associated with lymphedema in breast cancer patients: Should repeated ipsilateral arm infusions be avoided?

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Body: Background: Breast Cancer-Related Lymphedema (BCRL) is a chronic, iatrogenic condition that can occur after damage to the lymphatic system during surgery (sx) or radiation, precipitating edema of the arm, breast, or trunk. BCRL risk-reduction education is an essential component of clinical care, and practitioners often advise patients (pts) to avoid needle punctures on the treated arm when possible. There is, however, a lack of substantial scientific evidence to lessen patient distress. Considering the common use of chemotherapy (CT) agents in this population, we assessed whether repeated skin punctures on the ipsilateral arm for CT infusions increased the risk of BCRL compared to CT via central lines in a large, prospective cohort of breast cancer (BC) pts.

Methods: We prospectively screened 630 pts with unilateral (487) or bilateral (143) BC sx receiving neoadjuvant (NAC) and/or adjuvant CT (AdjCT) for arm lymphedema (defined as volume change $\geq 10\%$) at our hospital from 2005–16. Pts were measured with a perometer pre-operatively and at 3–7 month follow-up intervals. Clinicopathologic and treatment (tx)-related characteristics, including details on CT regimen and the method of intravenous (IV) CT administration [peripheral IV catheters (PIVCs), central venous access devices (CVADs), peripherally inserted central catheters (PICCs)] were obtained by chart review. Cox proportional hazard analyses were applied to ascertain the risk of BCRL associated with these factors.

Results: The median post-op follow-up was 44 months. Of the 630 pts, 40% underwent axillary lymph node dissection (ALND), 60% underwent sentinel lymph node biopsy (SLNB) or no nodal sx, 16% and 89% received NAC or AdjCT, respectively. CT was administered via PIVCs inserted in the hand/arm for 59%, via CVADs or PICCs for 26%, and via both PIVCs at least once and CVADs/PICCs for 15%. The 2-yr cumulative incidence of BCRL was 12% (95% CI 9.9-15.2%). Multivariable regression results indicated that pts with both peripheral IV infusions on the arm and implanted CVADs did not have a higher risk of BCRL (HR(95% CI)=1.4(0.6-3.6)) than pts who received CT via CVADs only (1.7(0.7-3.8)). The overall number of NAC ($p=0.24$; 0.9(95% CI 0.8-1.1)) or AdjCT cycles ($p=0.78$; 1.0(0.9-1.1)) was not associated with BCRL, nor was the number of peripheral IV infusions ($p=0.17$; 1.0(1.0-1.1)). BMI >30 ($p<0.0001$; 3.4(1.9-6.0)) and number of positive lymph nodes ($p=0.02$; 3.2(1.3-8.1)) were significantly associated with BCRL. Among those with PIVCs, pts with bilateral SLNB/ALND were more likely to develop BCRL than pts with unilateral sx ($p<0.01$; 5.0(1.9-13.4)). Only 38% of the 32 bilateral pts with BCRL received at least one peripheral IV infusion on their ipsilateral arm.

Conclusion: Results suggest that repeated skin punctures on the ipsilateral arm for CT infusions do not significantly increase the risk for BCRL compared to implanted CVADs, nor does the overall number of CT cycles. As survivors may be concerned about the risk of developing BCRL following sx and tx, healthcare practitioners should strive to mitigate pt worry during and well beyond the course of tx, educating pts about the lifestyle risk exposures for BCRL and precautionary guidelines not being definitive.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD4-04

Title: Effectiveness of myofascial techniques in addition to a standard physical therapy program as postoperative intervention for upper limb pain in breast cancer patients: A randomized controlled trial

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Body: Background: In addition to fatigue, pain is the most frequent and persistent symptom following cancer and cancer treatment. Despite the effectiveness of several postoperative physical therapy modalities, many patients after breast cancer still have pain at the upper limb region at short and long term. Several authors already recommended the use of myofascial therapy. Therefore, the aim of this study was to investigate short and long term effects of myofascial therapy, in addition to a standard physical therapy program, as postoperative intervention for upper limb pain after breast cancer surgery.

Methods: Randomized controlled trial with assessor and patient blinding and intention-to-treat analysis. One hundred forty-seven consecutive patients with breast cancer and unilateral axillary surgery were included. All participants received a standard physical therapy program starting immediately after surgery during 4 months. The intervention group received additionally 8 sessions of myofascial therapy on weekly basis from 2 up to 4 months after surgery. The control group received 8 sessions of a placebo intervention in addition to the same standard physical therapy program. Primary outcomes were prevalence rate of pain (i.e. pain at the operated upper limb region during the past week), maximal pain intensity at this region (Visual Analogue Scale (VAS) (0-100)), pressure hypersensitivity (pressure pain thresholds (kg/cm²) (PPT)) and pain quality (McGill Pain Questionnaire). All measurements were performed at 2 (=baseline), 4, 9 and 12 months post-surgery. Analysis of covariance (ANCOVA) were performed to correct for differences at baseline (i.e. 2 months after surgery). Additionally, effect size is given by means of relative risk reduction (RRR) and mean differences and their 95% confidence interval.

Results: Both groups were comparable at baseline. At 4, 9 and 12 months post-surgery, prevalence rates of pain were comparable between the intervention and control group (40%, 53%, 49% versus 41%, 47%, 51%, p=1.000, p=0.508 and p=0.868 and RRR of 3.2% (-0.44 - 0.35), -13% (-0.57 - 0.18) and 2.7% (-0.35 - 0.30), respectively). Pain intensity was comparable between the intervention group and control group as well. PPT of the Upper Trapezius muscle was significantly higher in the intervention group at 4 months (4.86 (2.43) versus 3.69 (2.04) kg/cm², p=0.012; mean difference -1.2 (-1.9 – 0.4) kg/cm²). PPT of the Supraspinatus muscle was significantly higher in the intervention group at 4 months (4.54 (2.1) versus 3.8 (1.85) kg/cm², p=0.021; mean difference -0.7 (-1.4 – 0.1) kg/cm²) and at 9 months (3.92 (1.7) versus 3.39 (1.61) kg/cm², p=0.040; mean difference -0.5 (-1.1 – 0.0) kg/cm²). No significant differences in pain quality were found between groups.

Conclusion: Myofascial therapy, in addition to a standard physical therapy program, has beneficial effect as postoperative intervention for pressure hypersensitivity of upper limb muscles after breast cancer surgery. No beneficial effect on pain prevalence rate, pain intensity and pain quality was found.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD4-05

Title: Survivorship care planning is associated with breast cancer survivors' reported quality and coordination of care

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Body: Primary goals of cancer survivorship care planning (SCP) include the assessment of ongoing risks for late effects and the coordination of health care. SCP strives to improve communication and coordination between survivors, health-care, and supportive care providers. Results on the effectiveness of SCP have been mixed. We report on the relationship between self-reported indicators of care with two essential elements of SCP: receipt of a written treatment summary and written instructions for routine cancer check-ups.

Methods: The Greater Plains Collaborative Clinical Data Research Network conducted the Share Thoughts on Breast Cancer survey across 8 cancer-care delivery sites across the Midwest. Participants were women age 18 years and older who had completed treatment for noninvasive or invasive (but not metastatic) breast cancer diagnosed from 1/1/2013 to 5/1/2014. Women were excluded if diagnosed with lobular carcinoma in situ, had previously been diagnosed with cancer per tumor registry records, did not report having cancer-directed surgery, or did not respond to questions about survivorship care planning. Logistic regression was used to examine characteristics and outcomes associated with a three-category SCP summary variable: (a) received both a written treatment summary and instructions for follow-up care; (b) received only one of these elements; or (c) received neither element. Adjustment variables included age at diagnosis, race, marital status at diagnosis, education, history of smoking, number of comorbidities, self-report that one health professional coordinated cancer care, type of surgery, and treatment with chemotherapy, radiation or hormones, and practice site.

Results: Of the 1148 survivors meeting study criteria, 485 (42.2%), 420 (36.6%), and 243 (21.2%) reporting receiving both SCP elements, one element, or no element, respectively. Several factors were associated with receiving elements of SCP: Those who reported having a single health professional who coordinated their cancer care were twice as likely to receive both elements of the SCP vs neither element (OR=2.3; 95% CI 1.6-3.2). Of patients who received both SCP elements, 88% reported excellent/very good quality of care compared to 73% who received neither element ($p = .001$). Respondents who reported always knowing who to ask questions about their cancer were far more likely to have received both elements vs neither element (OR=10.1; 95% CI 5.2-19.4). No association was observed between SCP and cancer care delivery site.

Conclusions: Breast cancer survivors who reported receiving a written summary of treatment and instructions for follow-up care reported better quality of cancer care, and they were also more confident about how to find answers to cancer related questions. Survivors who receive SCP were also more likely to have a single health professional coordinating their cancer care, potentially illustrating how SCP may fit into overall care processes.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD4-06

Title: Investigation of the recurrence dynamics of breast cancer (BC) according to the body mass index (BMI)

Biganzoli E, Desmedt C, Fornili M, Three-arms Investigators, de Azambuja E, Di Leo A, Sotiriou C, Piccart M and Demicheli R. University of Milan, Istituto Nazionale Tumori, Milan; Institut Jules Bordet- Université Libre de Bruxelles; The 'Three-Arms' Investigators and Ospedale Prato.

Body: Background:

In cancer follow-up (FU), in addition to the evaluation of disease free and overall survival probabilities, there is a fundamental need of assessing the recurrence dynamics. In BC as well as in other cancers, the hazard function for first recurrence presents multiple peaks, with a first major peak occurring before three years of FU. Although the baseline risk is modulated by known prognostic factors with possible time dependent effects such as the estrogen receptor (ER), so far no other factor proved to disentangle this multi-peak behavior. Here, we postulated that adiposity, which is closely related to a state of hyperinsulinemia and chronic inflammation, and reflected by increased patient's BMI, could influence the recurrence dynamics.

Material and methods:

In this study 777 patients with early node-positive BC from a phase III randomized clinical trial were considered (Piccart *et al.* JCO 2001). The trial compared intermediate or full doses of epirubicin–cyclophosphamide with cyclophosphamide, methotrexate and 5-fluorouracil. BMI was calculated using the WHO classification and was available for 734 patients, of whom 27(4%) were underweight, 377(51%) normal, 213(29%) overweight and 117(16%) obese. Underweight and normal patients were grouped together. Disease free survival (DFS), loco-regional and distant recurrence endpoints were considered. Median FU-time was 15.4 years. Cox regression analysis was performed, adjusting for standard clinico-pathological variables and treatment. Piecewise exponential models with cubic natural and regularized tensor product splines were carried out to estimate the hazard function according to categorical and continuous BMI, respectively.

Results:

Older age at diagnosis, postmenopausal status, and increased tumor size were significantly associated with increased BMI. Adjusted Cox models supported the association between overweight and disease recurrence (HR=1.39; 95%CI=1.05-1.84) as well as distant metastases (HR=1.41; 95%CI=1.01-1.97). There was no evidence of association for loco-regional recurrences. We observed a multi-peak behavior of distant recurrences for all BMI categories. Although, there was no shift of the first peak of recurrences according to BMI categories, occurring at ~2.5 years of FU, a major increase in peak heights for the overweight and obese patients was evident. Obese patients showed a sharper first peak. When considering the three BMI categories according to the ER-status, we observed the worst prognosis for overweight ER-negative patients, as well as different recurrence patterns (Table).

	Normal	Overweight	Obese
ER-positive	Main recurrence peak culminating at 2 years, with multiple smaller peaks later	One broad peak around 3 years and one smaller around 10 years	One narrow peak at 2 years and one smaller around 10 years
ER-negative	Similar pattern to ER-positive BC, with however major increase in height of first peak	One broad peak around 3 years, with a major increase in height compared to ER-positive BC	One peak at 2-3 years

Conclusion:

This is the first study to show that both the ER-status of the tumor and the patient's BMI at diagnosis are influencing the recurrence dynamics related to BC dormancy.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD4-07

Title: Are aromatase inhibitors associated with higher myocardial infarction risk in breast cancer patients? A Medicare population study

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Body: Purpose: Aromatase Inhibitors (AIs) are the current standard of care for post-menopausal adjuvant endocrine breast cancer therapy either following or in place of tamoxifen. While their short-term side effect profile was favorable in most studies, findings were mixed regarding cardiotoxicity, and cardiac outcome definitions were not consistent across studies. Given the five or more year duration of adjuvant endocrine therapy, risk of cardiotoxicity in older patients with pre-existing comorbidities remains a particular concern. We examined Myocardial Infarction (MI) as the cardiac outcome in subjects who received AIs vs. tamoxifen in a cohort of Medicare-based breast cancer survivors.

Patients and Methods: We identified women age ≥ 67 years diagnosed with breast cancer from 6/30/2006 to 6/01/2008 in the Surveillance, Epidemiology, and End Results-Medicare (SEER) database, with the following eligibility criteria: stage I-III breast cancer, continuous enrollment in Medicare Parts A and B for 24 months prior to the diagnosis and Part D enrollment for one month after the breast cancer diagnosis to the end of follow up (12/31/2012), adjuvant endocrine therapy (tamoxifen or AI fill) within 12 months after diagnosis. The main study outcome was the time to first diagnosis of MI after initiation of AIs or tamoxifen. MI was defined precisely by ICD9 and ICD10 codes relating both to incidence and death from MI. We developed and assigned stabilized inverse proportion weights to balance the groups, and performed a Fine and Gray hazards model for the outcomes of MI and death for the treatment groups of AIs vs tamoxifen.

Results: Of the cohort of 5,648 women, 4,690 were treated with AIs and 958 with tamoxifen; a total of 251 patients developed and/or died of MI during the study period while 476 died of other causes. The Fine and Gray Model results in a hazard ratio (HR) were not significantly different from one for weighted AI vs Tamoxifen groups [HR=1.01, C.I. 0.72-1.42]. Covariates which significantly affected the risk of MI were previous diabetes, prior other heart disease, prior congestive heart failure, prior MI, and prior peripheral vascular disease. Other covariates included in the weighted model, were age, American Joint Committee on Cancer (AJCC) cancer stage, chemo, Estrogen and progesterone receptor status, low income subsidy, marital status, prior hypertension, prior stroke, per capita income, race, radiation, SEER region, and urbanization.

Conclusions: The occurrence of MI is very low in this cohort (4.4%), reassuring the clinicians that the older adults with comorbidities may not be at a higher risk of MI with adjuvant endocrine therapy. However, the confidence interval for the hazard ratio of AIs vs Tamoxifen is very wide, indicating that a larger sample may be needed for the power of the study to be conclusive.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD4-08

Title: Efficacy of compression therapy using surgical gloves for nanoparticle albumin-bound-paclitaxel-induced peripheral neuropathy: A phase II multicenter study by the Kamigata breast cancer study group

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Body: PURPOSE: Chemotherapy-induced peripheral neuropathy (CIPN) is an adverse effect of many commonly used chemotherapeutic agents, including taxanes. However, there is currently no established effective prophylactic management for CIPN. Thus, we investigated the efficacy of using surgical glove (SG) compression therapy to prevent nanoparticle albumin-bound-paclitaxel (nab-PTX)-induced peripheral neuropathy.

PATIENTS AND METHODS: Patients with primary and recurrent breast cancer who received 260 mg/m² of nab-PTX were eligible for this case-control study. The patients wore two SGs of the same size, that is, one size smaller than the size that fit, on their dominant hand for 90 minutes. They did not wear SGs on the non-dominant hand, which served as the control hand. Peripheral neuropathy was evaluated at each treatment cycle using Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 and the Patient Neurotoxicity Questionnaire (PNQ). The temperatures of each fingertip of the compression SG-protected and control hands were measured by using thermography.

RESULTS: Between August 2013 and January 2016, 43 patients were enrolled, and 42 were evaluated. As shown in Table 1, the overall occurrence of [ge]grade 2 sensory and motor peripheral neuropathy according to the CTCAE was significantly lower in the SG-protected hands than in the control hands (76.1% vs. 21.4% and 57.1% vs. 26.2%, respectively, p [lt] 0.0001). The PNQ results showed that the incidence of [ge]grade 4 neuropathy was significantly higher in the control hands than in the SG-protected hands in terms of both sensory and motor neurotoxicity (p [lt] 0.0001, Table 2). As the treatment cycles of nab-PTX increased, the mean CTCAE and PNQ grades of the control hands gradually increased. However, the SG-protected hands maintained significantly lower mean grades than the control hands over time (p [lt] 0.0001).

No patients withdrew from this study because they could not tolerate the compression from the SGs. The mean temperature of each fingertip significantly decreased (1.42[ndash]2.60 [deg]C) in the SG-protected hands compared to in the control hands.
CONCLUSIONS: SG compression therapy appears effective for reducing nab-PTX-induced peripheral neuropathy. The nab-PTX exposure to the peripheral nerve may be decreased because the SG decreases microvascular flow to the fingertip.

Table 1: Comparison of the overall occurrences of the different grades of peripheral neuropathy according to CTCAE version 4.0 between the compression surgical glove-protected hands and control hands

CTCAE v.4.0 Grade	Sensory		Motor	
	Surgical Glove	Control	Surgical Glove	Control
0	12	4	18	7
1	21	6	13	11
2	9	24	11	16
3	0	8	0	8

4	0	0	0	0
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Table 2: Changes in the overall occurrence of the Patient Neurotoxicity Questionnaire (PNQ) grade with surgical glove compression therapy

PNQ Grade	Sensory		Motor	
	Surgical glove	Control	Surgical glove	Control
1	9	4	20	9
2	23	5	12	11
3	7	17	9	12
4	3	16	1	10
5	0	0	0	0

2016 San Antonio Breast Cancer Symposium

Publication Number: S3-01

Title: IMENEO: International MEta-analysis of circulating tumor cell detection in early breast cancer patients treated by NEOadjuvant chemotherapy

Bidard F-C, Michiels S, Mueller V, Riethdorf S, Esserman LJ J, Lucci A, Naume B, Horiguchi J, Gisbert-Criado R, Sleijfer S, Toi M, Garcia-Saenz JA A, Hartkopf A, Generali D, Rothe F, Smerage J, Muinelo L, Stebbing J, Viens P, Magbanua M, Hall CS S, Engebråtenm O, Takata D, Vidal-Martínez J, Onstenk W, Fujisawa N, Diaz-Rubio E, Taran F-A, Cappelletti MR, Ignatiadis M, Name N, Proudhon C, Wolf D, Bowman Bauldry J, Borgen E, Nagaoka R, Carañana V, Kraan J, Maestro M, Brucker SY, Weber K, Reyat F, Amara D, Gopalkrishna Karhade M, Ruud Mathiesen R, Tokiniwa H, Llombart-Cussac A, D'Hollander K, Cottu P, Park JW W, Loibl S, Pierga J-Y and Pantel K. Institut Curie, Paris, France; Gustave Roussy, Villejuif, France; Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; University of California at San Francisco, San Francisco, CA; MD Anderson Cancer Center, Houston, TX; Oslo University Hospital, Oslo, Norway; Gunma University Hospital, Gunma, Japan; Hospital Arnau de Vilanova, Valencia, Spain; Erasmus MC Cancer Institute, Rotterdam, Netherlands; Kyoto University Hospital, Kyoto, Japan; Hospital Clinico San Carlos, Madrid, Spain; University of Tuebingen, Tuebingen, Germany; University of Trieste, Cremona, Italy; Institut Jules Bordet, Brussels, Belgium; University of Michigan Comprehensive Cancer Center, Ann Arbor, MI; Complejo Hospitalario Universitario, Santagio de Compostella, Spain; Imperial College, London, United Kingdom; Institut Paoli Calmette, Marseilles, France; German Breast Group, Neu-Isenburg, Germany and IDDI, Louvain La Neuve, Belgium.

Body: Background

We performed an international meta-analysis of individual patient data to assess the clinical validity of circulating tumor cell (CTC) count in non-metastatic breast cancer (BC) patients (pts) treated by neoadjuvant chemotherapy (NCT).

Methods

A protocol pre-specified the study objectives. We performed a literature & abstracts search up to Dec 2014, then contacted all centers deemed to have eligible data (published or not): early BC pts treated with NCT with CTC count by CellSearch®. The primary endpoint was overall survival (OS); secondary endpoints included distant disease-free survival (DDFS), locoregional relapse-free interval (LRFI) and pathological complete response (pCR). Non-overlapping CTC time points were: baseline (5-0 weeks before NCT), 1-8 weeks after NCT start, 5-0 weeks before surgery and 1-52 weeks after surgery. We used Cox regression models, stratified by study, and the landmark method to establish the prognostic value of CTC count/changes during treatment and survival.

Results

We collected 2,156 individual pt data from 21 studies and 16 centers worldwide.

With $\geq 1/\geq 2/\geq 5$ CTC/7.5ml as thresholds, CTC positivity rate was 25/13/6% at baseline, 17/6/3% after NCT start, 15/5/1% before surgery and 11/4/1% after surgery (decrease, $p < 0.0001$). Before NCT, ≥ 1 CTC was found in 19%, 22%, 24%, 29% and 41% of cT1, T2, T3, T4a-c and T4d BC, respectively ($p < 0.0001$) and was also marginally associated with hormone-receptors negativity ($p = 0.04$). Later CTC detection rates were not associated with any of the baseline characteristics. pCR (assessed in 2,072 pts; ypT0/isN0 used as pCR definition in 92% of pts) was observed in 24% of pts but was not associated with CTC count, at any time point.

301, 418 and 157 events were reported for OS, DDFS and LRFI, respectively. In univariate analyses, ≥ 1 CTC at baseline was a prognostic factor for OS (HR=2.6 [1.9-3.4], $p < 0.0001$), DDFS (HR=2.4 [1.9-3.1], $p < 0.0001$) and -importantly- for LRFI (HR=1.8 [1.2-2.7], $p = 0.001$). Similar results were obtained using other thresholds (≥ 2 & ≥ 5 CTC) and/or later time points (after NCT start, before surgery). There was no interaction between the prognostic impact of CTC count and tumor subtypes. Although rare, pts with persistently elevated CTC count (≥ 1 CTC) before NCT and before surgery (5% of pts) had a worse OS than patients with persistently null CTC count (HR=6.2 [3.4-11], $p < 0.0001$).

Finally, in multivariate analyses, baseline CTC detection (whatever the CTC threshold used : $\geq 1/\geq 2/\geq 5$ CTC) was an independent prognostic factor for OS, DDFS and LRFI, together with pCR, cT, cN and tumor subtype, (e.g. for OS: CTC ≥ 2 HR=4.2 [3.0-5.9] $p < 0.0001$, No pCR HR=6.2 [3.7-11] $p < 0.0001$, cT4d HR=2.6 [1.1-6.6] $p = 0.02$, cN+ HR=1.7 [1.2-2.4] $p = 0.003$, triple negative BC HR=3.2 [2.1-5.1]). Similar results were obtained with later time points (after NCT start, before surgery).

Conclusions

Our study demonstrates with the highest level of evidence that CTCs are a prognostic biomarker in early BC treated by NCT. This impact was independent to that of pCR and was observed on OS, DDFS and also -for the first time- on LRFI. CTC count can usefully complement standard prognostic factors and pCR to improve the prognostication of early BC pts.

2016 San Antonio Breast Cancer Symposium

Publication Number: S3-02

Title: Plasma microRNA levels for predicting therapeutic response to neoadjuvant treatment in HER2-positive breast cancer: Results from Neo-ALTTO

Di Cosimo S, Appierto V, Tiberio P, Verderio P, Pizzamiglio S, Bottelli S, Iorio M, Baselga J, Piccart M, Huober J, Brase J, de la Pena L, Fumagalli D, de Azambuja E, de Braud F and Daidone MG. Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy; Memorial Sloan Kettering Cancer Center, New York; Université Libre de Bruxelles, Brussels, Belgium; Institut Jules Bordet, Brussels, Belgium; SOLTI Breast Cancer Research Group, Barcelona, Spain; Breast European Adjuvant Study Team, Brussels, Belgium; Universitäts-Frauenklinik, Ulm, Denmark and Novartis Pharmaceuticals, Basel, Switzerland.

Body: Background A significant portion of breast cancer patients treated in the neoadjuvant setting does not achieve a pathological complete response (pCR) and has an increased risk of relapse after surgery. The current lack of reliable predictors of response to guide patient therapy in clinical practice requires the identification of predictive biomarkers that could discriminate between responders and non-responders. The purpose of this study was to investigate the potential of circulating microRNAs (miRNAs) as novel non-invasive predictive biomarkers of response to neoadjuvant treatment in HER2-positive breast cancer patients enrolled in the Neo-ALTTO study. **Methods** We performed a longitudinal miRNA monitoring of the well-annotated and high-quality plasma samples of patients treated with neoadjuvant lapatinib, trastuzumab or their combination. Before profiling, the overall plasma samples were randomly splitted in two sets, namely the training set and the testing set, with distribution of timing of plasma collection, arm of treatment, rate of pCR, and tumor characteristics resembling that of the entire Neo-ALTTO patient population. To this end a PCR-based high-throughput approach was firstly employed in the training set. After a proper normalization step, for each treatment arm and time-point, a panel of potential miRNAs associated to pCR was identified by resorting to univariate approaches. Multivariate penalized logistic regression models were implemented thereafter, in order to identify specific signatures for each time-point and treatment arm. Finally, the predictive capability of the aforementioned signatures was evaluated in the testing set. **Results** Plasma miRNA profiles were available for 435 of the 455 (96%) patients enrolled in the Neo-ALTTO study. In details, miRNA levels were analyzed in 141, 151 and 143 patients assigned to neoadjuvant trastuzumab, lapatinib, and their combination, respectively, at baseline and after 2 weeks of treatment. A total of 30 miRNAs (including both normalizers and specific miRNAs) were identified and 6 signatures were found predictive of pCR in terms of Area Under the ROC Curve (AUC) in the training population. The predictive capability of 4 of 6 of the identified signatures was confirmed in the testing set, specifically: lapatinib at baseline AUC (95%CI) = 0.86 (0.73-0.98) and after two weeks AUC (95%CI) = 0.71 (0.55-0.86), trastuzumab after two weeks AUC (95%CI) = 0.81 (0.70-0.92), and lapatinib + trastuzumab after two weeks AUC (95%CI) = 0.67 (0.51-0.83). Of note, the predictive value of the signature of trastuzumab after two weeks was confirmed after adjustment for hormonal receptor status - training set AUC (95%CI) = 0.90 (0.80-0.99), testing set, AUC (95%CI) = 0.84 (0.74-0.94). **Conclusion** These findings provide the first evidence of the potential of circulating miRNAs to predict treatment response in HER2-positive breast cancer patients treated with neoadjuvant therapy. Results obtained in the trastuzumab arms are of special value as in women with unfavorable miRNA signature it is anticipated an unfavorable response to paclitaxel plus trastuzumab after just 2 weeks of treatment. Data on correlation with elapse free survival will be presented at the meeting.

2016 San Antonio Breast Cancer Symposium

Publication Number: S3-03

Title: PAM50 intrinsic subtype as a predictor of pathological complete response following neoadjuvant dual HER2 blockade without chemotherapy in HER2-positive breast cancer: First results of the PAMELA clinical trial

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Body: Background: Prior neoadjuvant studies in HER2+ breast cancer have shown that dual HER2 blockade without chemotherapy achieves pathological complete responses (pCR) rates of 6-36% (TBCRC006/TBCRC023/NeoSphere). However, a major challenge today is how to select prospectively patients who will derive the maximum benefit from dual anti-HER2 therapies without chemotherapy. In this context, we and others have previously shown that HER2+ disease is biologically heterogeneous and composed of all the intrinsic molecular subtypes (Luminal A, Luminal B, HER2-enriched [HER2-E] and Basal-like). Among them, the HER2-E subtype shows the highest activation of the EGFR/HER2 pathway.

Methods: PAMELA (NCT01973660) is a non-randomized, open-label, multicentric, prospective translational research study in stage I-IIIa HER2+ breast cancer designed to evaluate the ability of the PAM50 intrinsic subtypes to predict pCR in the breast (pCRB; *in situ* allowed) following 18 weeks of neoadjuvant lapatinib and trastuzumab). Patients with HR+ disease received letrozole (if postmenopausal) or tamoxifen (if pre-menopausal). The primary objective was to compare the pCRB rates of the HER2-E versus the non-HER2-E subtypes in the intent-to-treat population. The study was planned with a power of 95% at a significance level of 0.05 to detect an absolute relative difference in pCRB rates between the two groups of 27% (i.e. 35% in HER2E and 8% in non-HER2-E). Day-15 formalin-fixed, paraffin-embedded tissue samples were prospectively collected and gene expression profiled using the nCounter platform. The intrinsic subtypes were identified using the research-based PAM50 predictor (Parker JCO 2009).

Results: A total of 151 patients were recruited (n=77 HR+ and n=74 HR-). Patient characteristics were: mean age (55 years), mean tumor size (2.84 cm), negative axilla (63.5%) and postmenopausal (60.2%). At baseline, intrinsic subtype distribution was: HER2-E (n=101, 66.9%), Luminal A (n=22; 14.6%), Luminal B (n=16; 10.6%), Basal-like (n=9; 6%) and Normal-Like (n=3; 2%). The overall pCRB was 30.5% (46/151), 18.2% in HR+ disease and 43.2% in HR- disease. Five patients (3.3%) presented progressive disease. Rates of pCRB in HER2-E and non-HER2-E subtypes were 40.6% and 10.0% (p<0.0001), respectively. HER2-E subtype predicted pCRB independently of HR status. Within HR+ disease, pCRB rates were 31.6% in HER2-E subtype and 5.3% in non-HER2-E subtype (p=0.006). Within HR- disease, pCRB rates were 46.0% in HER2-E subtype and 27.3% in non-HER2-E subtypes (p=0.331). At Day-15, the majority of tumors became Normal-like (48.9%) or Luminal A (27.5%). Rates of pCRB were 46.9% in Normal-like tumors and 11.9% in non-Normal-like tumors when evaluated at day-15 (p<0.0001).

Conclusions: The PAMELA trial met its primary endpoint. PAM50 HER2-E subtype identifies patients with HER2+ disease likely to derive a large benefit from dual anti-HER2 therapies +/- endocrine therapy, especially in HER2+/HR+ disease. In addition, early changes in gene expression indicative of a reduction of tumor cellularity are predictive of pathological complete response at surgery.

2016 San Antonio Breast Cancer Symposium

Publication Number: S3-04

Title: Primary analysis of PERTAIN: A randomized, two-arm, open-label, multicenter phase II trial assessing the efficacy and safety of pertuzumab given in combination with trastuzumab plus an aromatase inhibitor in first-line patients with HER2-positive and hormone receptor-positive metastatic or locally advanced breast cancer

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Body: Background:

Preclinical and clinical evidence suggest that cross-talk between HER2 and estrogen receptor signaling pathways in breast cancer (BC) contributes to treatment resistance (Kaufman *et al. J Clin Oncol* 2009; Arpino *et al. J Natl Cancer Inst* 2007). First-line pertuzumab (P), in addition to trastuzumab (H) + docetaxel (T), significantly improved progression-free survival (PFS) and overall survival (OS) compared with H + T in patients (pts) with HER2-positive metastatic BC (MBC) (Swain *et al. N Engl J Med* 2015; Baselga *et al. N Engl J Med* 2012). PERTAIN (NCT01491737) is the first study to assess first-line P + H + an aromatase inhibitor (AI) ± induction taxane therapy in postmenopausal women with hormone receptor-positive, HER2-positive locally advanced (LA)/MBC.

Methods:

Pts with HER2-positive, hormone receptor-positive LA/MBC who had not received prior systemic therapy (except endocrine therapy) were randomized 1:1 to Arm A: P + H + AI or Arm B: H + AI. Study medication: P 840 mg loading dose followed by 420 mg every 3 weeks (q3w); H 8 mg/kg followed by 6 mg/kg q3w; anastrozole 1 mg daily (qd) or letrozole 2.5 mg qd. Induction T q3w or paclitaxel (PAC) weekly could be given for 18–24 weeks at the investigator's discretion before the start of endocrine therapy. Treatment was continued until disease progression or unacceptable toxicity. The primary endpoint was PFS, stratified by induction chemotherapy (yes/no) and time since adjuvant hormone therapy (<12 months, ≥12 months, no prior hormone therapy) and estimated using Kaplan–Meier methodology. Secondary endpoints included OS, objective response rate (ORR), duration of response (DoR), and safety and tolerability.

Results:

From February 2012 to October 2014, 129 pts were randomized to Arm A and 129 to Arm B (intent-to-treat populations; safety populations: 127 and 124, respectively) across 80 sites and 8 countries. Baseline demographics and disease characteristics were generally balanced between arms. Induction T and PAC were received by 42 (32.6%) and 32 (24.8%) pts in Arm A, respectively, and 37 (28.7%) and 31 (24.0%) pts in Arm B. Median PFS was 18.9 months in Arm A and 15.8 months in Arm B (HR 0.65; 95% CI 0.48–0.89; p=0.007). Median OS was not reached in either arm. ORR was 63.3% (95% CI 53.5–72.3) in Arm A and 55.7% in Arm B (95% CI 45.7–65.3; p=0.25). Median DoR was 27.1 months in Arm A and 15.1 months in Arm B (HR 0.57; 95% CI 0.36–0.91; p=0.02). All grade adverse events (AEs) occurred in 122 pts in each arm (96.1% in Arm A and 98.4% in Arm B); grade ≥3 AEs in 64 (50.4%) and 48 (38.7%) pts. The most common grade ≥3 AEs (≥5%; Arm A vs. Arm B) were hypertension (10.2% vs. 11.3%), diarrhea (7.1% vs. 2.4%), and neutropenia (3.1% vs. 6.5%).

Conclusions:

PERTAIN met its primary endpoint: P + H + AI is effective and well tolerated, and may offer a novel treatment option for pts with HER2-positive/hormone receptor-positive LA/MBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: S3-05

Title: Integrated analysis of multidimensional genomic data on CALGB 40601 (Alliance), a randomized neoadjuvant phase III trial of weekly paclitaxel (T) and trastuzumab (H) with or without lapatinib (L) for HER2-positive breast cancer

Tanioka M, Fan C, Carey LA A, Hyslop T, Pitcher BN N, Parker JA A, Hoadley KA A, Henry NL L, Tolaney S, Dang C, Krop IE E, Harris L, Berry DA A, Mardis E, Perou CM M, Winer EP P and Hudis CA A. University of North Carolina, Chapel Hill, NC; Alliance Statistics and Data Center, Duke University, Durham, NC; University of Michigan, Ann Arbor, MI; Dana Farber Cancer Institute, Boston, MA; Memorial Sloan Kettering Cancer Center, New York, NY; National Cancer Institute, Bethesda, MD; Alliance Statistics and Data Center, M.D. Anderson, Houston, TX and Washington University, St. Louis, MO.

Body: Background: RNA profiling and mutational analyses in CALGB 40601 (NCT00770809) found significant impact on pathologic complete response (pCR) rates from tumor (intrinsic subtype, p53 mutation) and microenvironmental (immune cell) features. Integrated analysis across platforms is needed to better understand the roles of these different factors with respect to response to HER2-targeted therapies.

Methods: We performed a comprehensive genomic analyses on pCR, defined as no invasive tumor in the breast, by integrating clinicopathological information with somatic mutation status, 422 segment-level DNA Copy Number Alterations (CNAs), and 510 gene expression signatures using mRNAseq and DNA exome sequencing from 213 pre-treatment tumors. Excluding 48 samples in the TL arm that was closed early due to futility, and 4 Normal-like tumors, the dataset consisted of 161 patients from TH and THL arms including 47 HER2-enriched (HER2E), 8 Basal-like, 54 Luminal A, and 52 Luminal B, all of whom received H. The main analysis was performed using the Elastic Net on multivariate logistic regression models for predicting pCR. The samples were divided into a training and a test set, then models were built to predict pCR by 10-fold cross-validation in the training set, then applying the best model onto the test set to construct ROC curves and evaluate prediction accuracy by calculating area under ROC (AUC). We also used the DawnRank, a network-based bioinformatics tool that integrates DNA and RNA data to identify driver genes, to find predictors of resistance to H-containing therapies.

Results: Among clinicopathological factors, clinical estrogen/progesterone receptor (ER/PgR) status and intrinsic subtype by PAM50 were statistically associated with pCR, but treatment arm (TH vs THL) and stage were not. In the Elastic Net analysis, the models incorporating either gene signatures (AUC: 0.724) or CNAs (AUC: 0.777) were more predictive of response than mutation status model (AUC: 0.635). Gene signatures and CNAs were further combined with either mutation status (AUC: 0.773), clinical ER/PgR status (AUC: 0.787) or ER/PgR status plus intrinsic subtype (AUC: 0.784). The combination with the highest AUC comprised gene signatures, CNAs, and ER/PgR status, and demonstrated that CNAs at Chromosome (Chr.) 6p, 10q22, or 11q23, the signature of Correlation to HER2E, and a T-cell signature, positively predicted pCR and that Luminal and PgR gene signatures were negative predictors. The CN gain of Chr.6p, which contains the HLA genes, predicted for pCR and was associated with higher expression of HLA genes and B cell / IgG signatures. The CN loss of Chr.11q23 including CD3D, CD3E, and CD3G was also identified by DawnRank as a region associated with resistance.

Conclusions: Tumor genetics (CNAs), tumor RNA subtype (HER2E, Luminal), and the microenvironment (immune cells) were independently predictive of response to H-containing therapies and biologically and clinically important for HER2-positive breast cancer, supporting integrated RNA- and DNA-based tumor assessments to clarify response to HER2-targeting.

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2016 San Antonio Breast Cancer Symposium

Publication Number: S3-06

Title: A phase III trial evaluating pCR in patients with HR+, HER2-positive breast cancer treated with neoadjuvant docetaxel, carboplatin, trastuzumab, and pertuzumab (TCHP) +/- estrogen deprivation: NRG Oncology/NSABP B-52

Rimawi MF F, Cecchini RS S, Rastogi P, Geyer, Jr CE E, Fehrenbacher L, Stella PJ J, Dayao Z, Rabinovitch R, Dyar SH H, Flynn PJ J, Baez-Diaz L, Paik S, Swain SM M, Mamounas EP P, Osborne CK and Wolmark N. NRG Oncology/NSABP (NSABP Legacy Trials Are Now Part of the NRG Oncology Portfolio); Baylor College of Medicine/Dan L Duncan Comprehensive Cancer Center, Houston; University of Pittsburgh; The University of Pittsburgh Cancer Institute; Massey Cancer Center, Virginia Commonwealth University; Kaiser Permanente Oncology Clinical Trials Northern California, Vallejo; Michigan Cancer Research Consortium NCORP; New Mexico Minority Underserved NCORP/ University of New Mexico Cancer Center; University of Colorado Cancer Center LAPS; Southeast Clinical Oncology Research (SCOR) Cancer Control Consortium NCORP; Metro Minnesota Community Oncology Research Consortium; Puerto Rico Minority Underserved NCORP, San Juan; Severence Biomedical Science Institute and Yonsei University College of Medicine; Lombardi Comprehensive Cancer Center, Georgetown University; UF Cancer Center at Orlando Health; Baylor College of Medicine/UT Health Science Center, San Antonio and Allegheny Health Network Cancer Institute.

Body: Background:

Preclinical evidence has shown that in xenograft models with estrogen receptor (ER)+/HER2+ breast cancer, signaling through the ER pathway can be enhanced in the presence of anti-HER2 treatment and lead to treatment resistance. Concurrent targeting of ER and HER2 has led to enhanced treatment efficacy and complete tumor disappearance. We hypothesized that targeting ER with endocrine therapy concurrently with chemotherapy plus dual HER2 inhibition will not be antagonistic and can overcome ER-mediated resistance and result in higher pCR as neoadjuvant treatment of ER+/HER2+ breast cancer. NRG Oncology/NSABP B-52 is a phase III, multicenter, randomized neoadjuvant therapy trial designed to determine whether the addition of estrogen deprivation to neoadjuvant therapy consisting of docetaxel, carboplatin, trastuzumab, and pertuzumab (TCHP+Est-Dep) yields a greater rate of pCR (breast and nodes) than TCHP alone.

Methods:

A total of 315 patients (pts) were randomly assigned between January 15, 2014 and March 17, 2016 to receive neoadjuvant therapy consisting of TCHP with or without estrogen deprivation therapy. Pts with locally advanced, hormone receptor-positive, HER2+ invasive breast cancer with no evidence of metastatic disease were eligible. Premenopausal women randomized to estrogen deprivation therapy received ovarian function suppression with goserelin (LHRH agonist) or equivalent plus an aromatase inhibitor (AI). Postmenopausal women received an AI. The determination of pCR (breast and nodes) was defined as the absence of any invasive component in the resected breast specimen and all resected lymph nodes following completion of neoadjuvant therapy. Following the intent-to-treat principle, the difference between the rates of pCR (breast and nodes) was tested using the binomial test for the difference between two proportions. The study was designed to have a statistical power of 80% to detect an increase in the pCR rate from 45% in the TCHP alone group to 60% in the TCHP+Est-Dep group.

Results:

The groups were balanced, with 57% clinically node positive and 50% premenopausal. Assessments for pCR were available from 308 of 315 randomized patients. The pCR (breast and nodes) for TCHP alone and TCHP+Est-Dep were 40.9% and 46.1%, respectively (p=0.36). The pCR (breast) were 44.2% and 47.4%, respectively (p=0.57). Grade 3/4 toxicities included diarrhea (23%, <1% vs 21%,0%) vomiting (8%, <1% vs 5%, 0%), and febrile neutropenia (5%, <1% vs 7%,1%) for TCHP vs TCHP plus estrogen deprivation.

Conclusion:

The addition of estrogen deprivation to neoadjuvant chemotherapy is not antagonistic. It improved pCR rates numerically, but the improvement was not statistically significant. The combination did not increase toxicity and may be a reasonable approach since all patients will receive endocrine therapy after neoadjuvant therapy. Correlative science studies including evaluation of residual cancer burden (RCB) and long-term outcomes will help define the role of estrogen deprivation in the treatment of HER2+ early breast cancer.

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2016 San Antonio Breast Cancer Symposium

Publication Number: S3-07

Title: Impact of radiotherapy on complications and patient-reported satisfaction with breast reconstruction: Findings from the prospective multicenter MROC study

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Body: Background: Patients considering both post-mastectomy radiation (RT) and reconstruction require robust information regarding the expected outcomes of different combinations of approaches in order to make preference-concordant decisions. Methods: In a prospective multicenter cohort study (the Mastectomy Reconstruction Outcomes Consortium, MROC, funded by NCI 1RO1CA152192) of women diagnosed with breast cancer at 11 institutions between 2012-15, we compared responses of 553 radiated and 1461 non-radiated pts who received different approaches to reconstruction. The primary dependent variables of interest were development of any breast complications (e.g. hematoma, wound infection) by one year post-reconstruction, along with satisfaction measured with the validated BREAST-Q instrument. Mixed-effects regression models assessed impact of reconstruction type and RT on the outcomes of interest. Covariate adjustment included reconstruction timing, age, extent of disease, bilateral vs unilateral treatment, chemotherapy receipt, nodal management, BMI, smoking, diabetes, race, ethnicity, education, employment, income, marital status, and hospital site.

Results: Median age was 49. Bilateral mastectomy was received by 45.6% of radiated and 53.3% of non-radiated pts ($p=0.002$). Autologous reconstruction was more commonly received by radiated pts (38.3% vs 25.1%, $p<0.001$). Immediate reconstruction was less common in radiated pts (82.6% vs 95.6%, $p<0.001$). By one year, at least one complication occurred in 28.8% of radiated pts (30.8% of implant pts and 25.5% of autologous pts) and 22.3% of non-radiated pts (20.4% of implant pts and 28.1% of autologous pts). Among pts with ≥ 2 years of follow up, a complication had occurred by 2 years in 34.1% of 331 radiated pts vs 22.5% of 946 non-radiated pts. Multivariable analysis showed immediate reconstruction, bilateral treatment, & higher BMI to be predictive of developing a complication by one year. RT effect differed by reconstruction type; RT was associated with 2.1 (95% CI = 1.45, 3.10) times higher odds of complication in implant pts, while showing no difference in autologous pts (OR=1.3, 95% CI = 0.76, 2.09). RT effect on patient outcomes also differed by reconstruction types. In implant pts, adjusted mean BREAST-Q satisfaction with breast scores were significantly lower in radiated pts than in non-radiated pts (51.5 vs. 58.0 at 1 year, $p<.001$; 48.9 vs. 59.8 at 2 years, $p<.001$), while satisfaction in autologous pts did not differ by radiation (61.3 in radiated vs. 63.5 in non-radiated at 1 year; 62.8 vs. 65.8 at 2 years). Similarly, in implant pts, satisfaction with outcomes was significantly lower in radiated versus non-radiated pts (66.5 vs. 70.8 at 1 year; $p=0.03$; 64.4 vs. 70.6 at 2 years, $p=0.03$), while there were no significant differences in autologous pts (72.7 vs. 75.2 in radiated vs. non-radiated at 1 year; 71.3 and 75.3 at year 2).

Conclusions: In the largest prospective multicenter study of outcomes of breast reconstruction to date, autologous reconstruction appears to yield superior patient-reported outcomes and lower risk of complications than implant-based approaches among patients receiving PMRT.

2016 San Antonio Breast Cancer Symposium

Publication Number: S3-08

Title: Radioactive seed localization versus wire guided localization of nonpalpable invasive and in situ breast cancer: A Danish multicenter randomized controlled trial

Langhans L, Tvedskov TF, Filtenborg, Klausen TL, Levin, Jensen M-B, Talman M-L, Vejborg I, Benian C, Roslind A, Hermansen J, Oturai PS, Sandor, Bentzon N and Kroman N. Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; Danish Breast Cancer Cooperative Group, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; Herlev Hospital, University of Copenhagen, Denmark, Herlev, Denmark; Herlev Hospital, University of Copenhagen, Herlev, Denmark and Herlev Hospital, University of Copenhagen, Herlev, Denmark.

Body: Background:

The current standard method for locating nonpalpable breast lesions is wire guided localization (WGL) despite several methodological difficulties. Radioactive Seed Localization (RSL) has been developed to reduce these difficulties. The aim of this randomized trial was to compare the rate of positive resection margins between RSL and WGL in patients with nonpalpable invasive breast cancer (IBC) or ductal carcinoma in situ (DCIS).

Material and Methods:

Patients with nonpalpable IBC or DCIS visible on ultrasound were randomized to either of the two localization methods. Primary outcome was margin status at the final pathological evaluation. According to Danish standard in the study period margins were defined positive if cancer cells were found < 2mm from the inked margin. Secondary outcomes were duration of the surgical procedure, weight of the excised specimen and patient's pain perception. χ^2 -test, Fisher's exact test and Wilcoxon rank-sum test, respectively, were used to test differences between groups. Level of statistical significance was set to 5%. The average activity of seeds used in the trial was 1.70 MBq (range 0.7-3.27).

Results:

413 cases representing 409 patients were randomized; 207 were allocated to RSL and 206 to WGL. 23 cases, who did not meet inclusion criteria, chose to withdraw, or had a change in surgical management, were excluded. The remaining 390 were included in the analysis. Patient, surgical and pathological characteristics between the two groups were alike, except for significantly more patients with DCIS in the WGL group (5.1% vs 0.5%).

Significantly more cases in the WGL group (9.7%) needed additional localization compared to the RSL group (2.1%) ($p=0.0014$). In all cases but one in the RSL group, the index lesion was removed.

Margins were positive in 23 cases (11.8%) in the RSL group compared to 26 cases (13.3%) in the WGL group. We were not able to detect a difference in margin status between the two groups ($p=0.65$). For IBC only, the number of positive margins was 22 (11.3%) in the RSL group and 21 (11.4%) in the WGL group ($p=0.997$). There was no difference between the two groups in the amount of tissue removed whether the analysis was done on the primary excision ($p=0.18$) or the total weight including intraoperative re-excisions ($p=0.33$). There was no difference in pain perception between the two groups whether patients who received local anesthesia were kept in the analysis ($p=0.28$) or excluded ($p=0.91$). Local anesthesia was used more frequently in the RSL group. Finally, there was no difference in the duration of the surgical procedure ($p=0.12$), the complication rate ($p=0.89$) or the identification rate for SN ($p=1.0$).

Conclusions:

We were not able to detect any differences considering positive margins, patient's pain perception or duration of the surgical procedure between the two localization methods. However, RSL offers a major logistic advantage, as the seed localization can be done several days before surgery without any risk or discomfort for the patient, with a low proportion of patients needing additional localization. So the RSL procedure has now been found preferable at our institutions.

2016 San Antonio Breast Cancer Symposium

Publication Number: S4-01

Title: A novel BRD4 inhibitor enhances endocrine therapy efficacy and circumvents endocrine-resistance in estrogen receptor-positive breast cancer models

De Angelis C, Nardone A, Cataldo ML, Fu X, Trivedi M, Yi S, Breckenridge D, Chamnsess GC C, Vitorino P, Osborne CK Kent and Schiff R. Lester & Sue Smith Breast Center, Baylor College of Medicine, Houston, TX; Dan L. Duncan Comprehensive Cancer Center, Baylor College of Medicine, Houston, TX; Baylor College of Medicine, Houston, TX; University of Houston College of Pharmacy, Houston, TX and Gilead Science, Foster City, CA.

Body: Background: The selective estrogen receptor (ER) down-regulator (SERD) fulvestrant (Ful) antagonizes ER activity and degrades ER protein in a dose-dependent manner in the preclinical and clinical settings, though its efficacy is limited by an incomplete abolition of ER protein levels. Therefore additional strategies are needed to achieve a more complete suppression of ER level and activity. The bromodomain-containing protein 4 (BRD4), a member of the BET family that is required both for *ESR1* gene expression and for ER-mediated gene transcription, represents an attractive therapeutic target for ER+ breast cancer (BC). Here, we investigated the efficacy of the novel BRD4 inhibitor GS-626510 (GS-6510) in a panel of ER+ BC parental and endocrine-resistant (EndoR) cell lines and in a patient-derived xenograft (PDX) model.

Materials and Methods: The effects of GS-6510 (25nM – 290nM) alone or in combination with endocrine therapies were tested in ER+ MCF7, T47D, and ZR75-1 cell lines, as well as in their derivatives made resistant to estrogen (E2) deprivation (EDR), tamoxifen (TamR), or Ful (FulR). Cell growth (by methylene blue) after 6 days of GS-6510 and protein levels (by Western blot) after 2 days of GS-6510 were assessed. The *in vivo* efficacy of GS-6510, Ful, and the combination was tested in the ER+ HCBx34 PDX model. The mRNA levels of genes associated with cell cycle, ER signaling, and endocrine-resistance were assessed using the NanoString platform.

Results: A dose-dependent inhibitory effect of GS-6510 was observed in all of the experimental settings. At a clinically relevant dose, GS-6510 reduced E2-stimulated cell growth and enhanced the efficacy of endocrine therapies in all parental cell lines. In the endocrine-sensitive HCBx34 PDX model, GS-6510 reduced tumor growth and, in combination with Ful, induced tumor regression and inhibited the expression of ER-dependent and cell cycle related genes including CCND1, MYC, and BCL2. Notably, the addition of GS-6510 to EndoR cell models that continue to rely on and express ER (MCF7 EDR and TamR) led to a substantial inhibition in cell growth (85%; 98%, respectively). Though the combination of GS-6510 and Ful was not associated with a significantly greater cell growth inhibition compared to GS-6510 alone in these models, a better suppression of ER levels was observed. Interestingly, GS-6510 also remained effective in EndoR models that exhibited an ER-independent growth, including all FulR lines, though its efficacy varied among the different cell lines and resistant derivatives.

Conclusion: Our findings suggest that the epigenetic regulator BRD4 is a suitable target for therapeutic intervention in ER+ BC. The anti-tumor efficacy of GS-6510 in endocrine sensitive and especially in ER-dependent EndoR models is worthy of further clinical investigation. The growth inhibitory effects observed in some of the ER-independent EndoR models suggests that additional genes/pathways involved in endocrine resistance could be affected by GS-6510. Identifying these pathways and determining their predictive role are needed to guide patient selection for future clinical trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: S4-02

Title: The Y537S ESR1 mutation is a dominant driver of distant ER-positive breast cancer metastasis

Fuqua SAW AW, Gu G, Rechoum Y, Gelsomino L, Dustin DJ J, Corona-Rodriguez A, Beyer AR R, Pejerrey SM M, Gao M, Tsimelzon A, Tian L, Zhang X, Nagi C and Ando' S. Baylor College of Medicine, Houston, TX; University of Calabria, Cosenza, Calabria, Italy and MD Anderson Cancer Center, Houston, TX.

Body: Background: Estrogen receptor (ESR1) mutations occur at a high frequency in metastatic breast tumors in patients treated with hormonal therapy in the metastatic setting. We do not know if these mutations are involved in metastasis.

Experimental design and methods: We generated ESR1 Y537S homozygous mutations using CRISPR Casp-9 technology. Treatment synergy was evaluated using Compusyn. Athymic mice were used in tumor xenograft studies. ChIP-Seq and transcriptome analyses were performed.

Results: We generated CRISPR ESR1 Y537S mutation homozygous knock-in clones and lentiviral stable pools in MCF-7 cells. Transcriptome profiling revealed elevated expression of Hallmark pathways, including EMT and estrogen-regulated gene expression. The EMT in mutant cells was associated with a switch from E-cadherin to vimentin, and increased expression of SNAIL and TWIST. Mutant cell growth was resistant to tamoxifen, but responsive to fulvestrant treatment. Synergistic treatment effects were observed with fulvestrant and everolimus or palbociclib. CRISPR Y537S mutant knock-in cells grown in the mammary fat-pad of athymic mice spontaneously metastasized to distant organs including the lung, intestine, and kidneys. In the presence of estrogen, there was no difference in the frequency of distant macrometastases between parental wild-type ER and CRISPR Y537S mutant ER mice. However, in the absence of estrogen, 80% of CRISPR Y537S mutant ER mice displayed overt distant macrometastases, but none were observed in parental wild-type ER mice ($p=0.04$). Interestingly, although CRISPR Y537S mutant ER tumors grown in the mammary fat-pad were unresponsive to tamoxifen treatment, tamoxifen significantly inhibited the growth of mutant tumors at the distant microenvironment (8-fold). Distant tumors retained ER expression and hormone sensitivity. Comparison of residual tamoxifen-treated metastatic tumors with tumors grown at the primary mammary fat-pad site using immunoblot analysis demonstrated significant reduction in estrogen-regulated gene expression, but no effect on the expression of biomarkers associated with EMT, suggesting a disconnect between EMT and distant metastasis in mutant cells. EMT genes were also identified as direct binding site targets in Y537S mutant cells compared with wide-type ER using ChIPSeq. We discovered that expression of the Y537S mutant was dominant, driving the growth of distant metastatic tumors when co-expressed with wild-type ER cells. A Y537S ER mutant-specific gene expression signature predicted poor disease-free survival of ER-positive patients using the METABRIC database, and lung-specific metastasis-free survival in a Memorial Sloan Kettering dataset.

Conclusion: The Y537S ER mutation is a driver of distant metastasis in ER-positive breast cancer cells. Although tamoxifen treatment was ineffective at reducing the growth of mutant cells grown at the primary site, it was effective at reducing distant metastasis. A Y537S ER mutant-specific gene expression signature predicted poor disease-free, and distant lung metastasis in ER-positive patients. Mutation status is a potential new predictive factor for hormone therapy of metastatic breast cancer patients on maintenance hormonal therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: S4-03

Title: Targeted and selective degradation of estrogen receptor (ER) alpha by PROTACs

Flanagan JJ J, Rossi AK K, Anderoli M, Willard RR R, Gordon D, Harling J, Churcher I, Smith I, Zinn N, Bantscheff M, Crews CM M, Crew A, Coleman KG G, Winkler JD D and Qian Y. Arvinas LLC, New Haven, CT; GSK Medicines Research Centre, Stevenage, United Kingdom and Yale University, New Haven, CT.

Body: ER α -positive breast cancers comprise approximately 80% of all newly diagnosed cases. Current treatment approaches targeting ER signaling include antagonizing and/or downregulating ER or reducing estrogen levels. Fulvestrant (fulvestrant) is the only clinically-approved agent that is both a potent ER antagonist and downregulator but has limitations given its pharmacokinetics and route of administration. Over the past several years, targeted ER therapies have focused on developing selective estrogen receptor downregulators (SERDs, i.e, GDC-0810, GDC-0927, AZD9496, RAD1901). The mechanisms involved in ER downregulation by SERD binding are not completely understood, but evidence suggests that conformational changes in the receptor upon ligand binding combined with specific co-regulator interactions destabilize the receptor making it a target for passive proteasomal degradation. We hypothesized that the complex ER pharmacology required for SERD-based passive degradation might be different across various ER-positive cell lines and that targeted degradation of the receptor by actively recruiting the ubiquitin-proteasome machinery would provide a better approach for reducing ER levels. To test this hypothesis, we developed potent molecules directed against ER using our pioneering technology proteolysis targeting chimeras (PROTACs). PROTACs are heterobifunctional molecules that actively recruit specific E3 ligases resulting in ubiquitylation and degradation of target proteins. When testing for ER degradation using several SERDs and ER PROTACs, we discovered that both fulvestrant and ER PROTACs provided robust degradation in all ER-positive lines (<1 nM 50% degradation; >90% reduction) whereas other SERDs did not degrade or only modestly degraded the receptor. Importantly, MCF-7 cells were uniquely sensitive to SERD-based degradation of ER compared to other cell lines. Subcutaneous administration of fulvestrant (1mpk) or ER PROTACs (10 mpk) reduced uterine ER alpha levels in immature rats (>65% reduction). PROTAC-mediated degradation of ER was also achieved in breast cancer xenografts. To further validate the PROTAC mechanism, incubation of ER-positive cells with ER PROTACs resulted in increased levels of poly-ubiquitylated ER α when compared to SERDs. Lastly, to demonstrate the specificity of PROTAC-mediated ER α degradation, we utilized a cellular expression proteomics-based approach to examine over 7,000 proteins. In this experiment, only ER α and several known proteins whose genes are regulated by ER α , were significantly reduced by PROTACs. It remains to be seen how the current class of investigational downregulators will perform in the clinic. More importantly, a better understanding of the therapeutic potential and benefit of degrading the receptor instead of inhibiting the receptor needs to be explored. To that end, we continue to develop and characterize novel ER PROTACs with the anticipation that targeted ER α degradation will provide a greater clinical benefit than receptor antagonism.

2016 San Antonio Breast Cancer Symposium

Publication Number: S4-04

Title: Tumor microenvironment of metastasis (TMEM) score is associated with early distant recurrence in hormone receptor (HR) positive, HER2-negative early stage breast cancer (ESBC)

Sparano JA A, Gray R, Oktay MH H, Entenberg D, Rohan T, Xue X, Donovan M, Peterson M, Shuber A, Hamilton D, D'Alfonso T, Goldstein LJ J, Gerlter F, Davidson N, Condeelis J and Jones J. Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; ECOG-ACRIN Research Group, Boston, MA; Albert Einstein College of Medicine, Bronx, NY; Mt. Sinai School of Medicine, New York, NY; MetaStat, Inc, Boston, MA; Weill Cornell Medical College, New York, NY; Fox Chase Cancer Center, Philadelphia, PA; Massachusetts Institute of Technology, Boston, MA and University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Body: Background: Metastasis is the primary cause of death in ESBC. We have shown in mouse models that a subpopulation of tumor cells expressing invasive Mena isoforms stream, form microanatomic structures ("TMEM") with endothelial cells and macrophages, intravasate into the circulation at TMEM sites, and metastasize (Harney et al. Cancer Discovery, 2015). Further, TMEM sites ("MetaSites") are identifiable in human ESBC, and "MetaSite score" [MS] is positively associated with distant recurrence in HR+/HER2- ESBC independent of clinicopathologic features, including IHC4 (Rohan et al. JNCI 2014). Here we determined the association between MS and recurrence in an independent ESBC cohort (E2197; NCT00003519).

Methods: We evaluated primary tumors from 600 patients (median followup 14.8 years) with ESBC (weighted % = 50% T1, 54% N0, 46% N1) treated with surgery and 4 cycles of adjuvant chemotherapy (AC or AT) and endocrine therapy. Grade, ER, PR, and HER2, and Oncotype DX Recurrence Score (RS) were evaluated in central labs (Badve et al. JCO 2008), and MS was determined in a CLIA-certified lab using an analytically validated, fully automated digital pathology/image analysis method that identifies Mena expressing tumor cells in direct contact with CD68+ macrophages and CD31+ endothelial cells (ie, "TMEMs", or "MetaSites"). The objectives were to determine the association between MS and distant relapse free interval (DRFI) and relapse free interval (RFI). Kaplan-Meier survival curves were used to estimate time-to-event distributions. Cox proportional hazards models were used to assess hazard ratio associated with MS while controlling for covariates, and allowing time-varying association with MS. Both Kaplan-Meier and Cox regression methods addressed stratified sampling by incorporating proper weights. All analyses were performed in R 3.2.3.

Results: MS ranged from 0-199; the weighted mean MS was lower in HR+/HER2- than TN (16.1 vs. 23.8, $p=0.001$) and HER2+ disease (26.2, $p=0.003$). MS was not associated with T or N status, and correlated poorly with RS ($r=0.29$). Proportional hazards models revealed a significant positive association between continuous MS and DRFI ($p=0.001$) and RFI ($p=0.00006$) in HR+/HER2- disease in years 0-5 (and by MS tertiles for DRFI [$p=0.04$] and RFI [$p=0.01$]), but not after year 5 or in TN or HER2+ disease. Proportional hazards models including clinical covariates (N0 vs. N1; T1 vs. T2; high vs. int. vs. low grade) also revealed significant positive associations for continuous MS with RFI ($p=0.04$), and borderline association with DRFI ($p=0.08$). Similar findings for MS (RFI $p=0.05$; DRFI $p=0.10$) were noted in a joint model including categorical RS (<18, 18-30, >30).

Conclusions: MS, a novel metastasis biomarker reflecting interaction between streaming and metastasizing tumor cells and microenvironment, provides prognostic information complementary to classical clinicopathologic features and RS in HR+/HER2- ESBC. Further evaluation is warranted in order to identify patients at highest risk of recurrence within 5 years most likely to benefit from adjuvant chemotherapy or novel therapies. (Supported by BCRF and NCI CA21115, CA180794, CA23318, CA66636, CA180820).

2016 San Antonio Breast Cancer Symposium

Publication Number: S4-05

Title: Expression of the DEK oncogene promotes M2 polarization and iron recycling in tumor associated macrophages

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Body: The chromatin-remodeling DEK oncogene is highly expressed in over 60% of breast cancers, regardless of sub-type. DEK over-expression correlates with poor clinical outcome, including decreased survival and chemotherapy resistance. Previously, using human breast cancer cell lines and the MMTV-Ron mouse breast cancer model, we have demonstrated that DEK expression promotes proliferation and metastasis and supports the breast cancer stem cell population. These functions were due, in part, through upregulated Wnt ligand expression and β -catenin activation, leading to paracrine and autocrine effects promoting tumorigenesis. To investigate additional drivers of DEK-induced tumorigenesis, we performed RNA-Sequencing on control and DEK over-expressing immortalized MCF10A cells. Gene ontology analysis identified several immune signaling processes. This is in alignment with the well-characterized role of DEK expression in the promotion of inflammatory diseases. We investigated the immune cell infiltration of tumors from MMTV-Ron/Dek^{+/+} and MMTV-Ron/Dek^{-/-} mice. Interestingly, we discovered that Dek-expressing tumors demonstrated decreased tumor associated macrophage (TAM) infiltration, where TAMs were largely limited to the periphery. Furthermore, these TAMs demonstrated an iron-recycling phenotype (M2 polarization), whereas TAMs from Dek-knockout tumors retained iron (M1 polarization). M1 macrophages are typically associated with inflammatory responses whereas M2 macrophages have been linked to tissue remodeling, angiogenesis, and tumor promotion. To investigate this further, we compared Dek^{+/+} and Dek^{-/-} bone marrow-derived macrophages (BMDM) and found no inherent difference in polarization. However, BMDM demonstrated different gene expression profiles when cultured in conditioned media from Dek-expressing murine breast cancer cell lines compared to isogenic Dek^{-/-} cells. BMDM exposed to conditioned media from Dek-expressing cancer cells demonstrated the same iron recycling phenotype observed *in vivo*, likely caused by increased expression of ferroportin, as well as increased expression of M2 markers CXCR4, VEGF, and an elevated ARG1^{hi}/NOS^{lo} population. We thus used a combination of genetic models and small molecule inhibitors to identify the mechanism of M2 polarization of BMDM exposed to Dek-expressing cancer cells. NRF2 transcription activity was eliminated as a possible mechanism for ferroportin regulation. However, small molecule inhibitors and gene expression analyses implicated secreted Wnt ligands and S100A8/A9 alarmins from the cancer cells, and downstream C/EBP transcriptional activity in the macrophages, as possible mechanisms of Dek-induced M2 polarization. Combined, this suggests that the poor clinical outcome of high DEK expressing breast cancers may be due to not only cell intrinsic factors, but also cell extrinsic factors, such as the tumor-promoting M2 polarization of tumor associated macrophages.

2016 San Antonio Breast Cancer Symposium

Publication Number: S4-06

Title: Biological effects of abemaciclib in a phase 2 neoadjuvant study for postmenopausal patients with HR+, HER2- breast cancer

Hurvitz S, Martin M, Fernández Abad M, Chan D, Rostorfer R, Petru E, Barriga S, Costigan TM Michael, Caldwell CW William, Nguyen T, Press M and Slamon D. University of California, Los Angeles, CA; Hospital General Universitario Gregorio Marañón, Madrid, Spain; Hospital Universitario Ramón y Cajal, Madrid, Spain; Cancer Care Associates, Redondo Beach, CA; UF Health Cancer Center at Orlando Health, Orlando, FL; Medical University Graz, Graz, Steiermark, Austria; Eli Lilly and Company, Madrid, Spain; Eli Lilly and Company, Indianapolis, IN and University of Southern California, Los Angeles, CA.

Body: Background:

Abemaciclib is a potent oral CDK4 and 6 inhibitor, which demonstrated evidence of clinical activity and an acceptable safety profile on a continuous dosing schedule either as a single agent (MONARCH 1; NCT02102490) or in combination with endocrine therapies in heavily pre-treated women with HR+ metastatic breast cancer (BC).^{1,2}

Methods:

neoMONARCH (NCT02441946) is a randomized, multicenter, open-label phase 2 neoadjuvant study comparing the biological effects of abemaciclib plus anastrozole vs abemaciclib monotherapy vs anastrozole monotherapy in women with early-stage HR+, HER2- BC. Patients (pts) were stratified by progesterone receptor status and tumor size and randomized 1:1:1 ([abemaciclib 150mg orally [PO] every 12 hours [Q12H] plus anastrozole 1 mg PO daily [QD]], [abemaciclib 150mg PO Q12H], and [anastrozole 1mg PO QD]). Each regimen was given for 2 weeks; followed by all pts receiving abemaciclib 150mg PO Q12H plus anastrozole 1mg QD for the subsequent 14 weeks. All pts received prophylactic loperamide during the first 28 days of abemaciclib therapy, then at the discretion of the investigator. Eligible pts included postmenopausal women with HR+, HER2- clinical Stage I breast tumor ≥ 1 cm in diameter, Stage II, Stage IIIA, or IIIB BC. The primary objective: compare biological activity by assessing the percent change from baseline value in Ki67 protein expression after 2 weeks of therapy with the three initial regimens. Clinical activity and safety of the subsequent 14 weeks of therapy of abemaciclib plus anastrozole are evaluated as secondary objectives at surgery. Exploratory objectives included assessment of changes in cell cycle mRNAs by Modaplex (QIAGEN) analyses and *PIK3CA* and *ESR1* mutational analysis in core biopsy samples.

Statistical methods:

The design provides 80% power to detect superiority of the combination vs anastrozole monotherapy, and 80% power to detect superiority of abemaciclib monotherapy vs anastrozole monotherapy, at a 1-sided alpha level of 0.1. Secondary and exploratory objectives will be presented as descriptive data.

Results:

Enrollment of 223 pts began August 2015 and was completed August 2016. At a 9 month interim analysis, abemaciclib, given either as monotherapy or in combination with anastrozole showed significantly ($p < 0.001$, $n = 64$) greater suppression of Ki67 after 14 days of dosing than anastrozole alone. The safety profile of the combination differed from that previously reported for abemaciclib 200mg BID monotherapy in the MONARCH 1 study¹ and the Phase Ib² with reduced incidence of diarrhea and hematologic events. Change in proliferation gene mRNAs after 2 weeks of treatment ($n = 38$), in both tumor and blood, appeared to correlate with change in Ki67 expression, with a greater reduction in the abemaciclib-containing arms. Updated data will be presented including safety data for all 223 pts, molecular data on approximately 150 pts who are evaluable for change in Ki67 and mRNA expression at 2 weeks and approximately 100 pts evaluable for clinical efficacy, final Ki67 and RNA expression at surgery.

References:

1. Dickler, M.et al., *ASCO* abstract #510 (2016).
2. Goetz, M.et al., *SABCS* abstract #P4-13-25 (2015).

2016 San Antonio Breast Cancer Symposium

Publication Number: S4-07

Title: BELLE-3: A phase III study of buparlisib + fulvestrant in postmenopausal women with HR+, HER2-, aromatase inhibitor-treated, locally advanced or metastatic breast cancer, who progressed on or after mTOR inhibitor-based treatment

Di Leo A, Seok Lee K, Ciruelos E, Lønning P, Janni W, O'Regan R, Mouret Reynier M-A, Kalev D, Egle D, Csozsi T, Bordonaro R, Decker T, Tjan-Heijnen VC, Blau S, Schirone A, Weber D, El-Hashimy M, Dharan B, Sellami D and Bachelot T. Ospedale Misericordia e Dolce, Prato, Italy; National Cancer Center, Gyeonggi-do, Republic of Korea; Hospital Universitario 12 de Octubre, Madrid, Spain; Haukeland University Hospital, Bergen, Norway; Universitätsklinikum Ulm, Ulm, Germany; University of Wisconsin, Madison, WI; Centre Jean Perrin, Clermont-Ferrand, France; Medical University of Varna, Varna, Bulgaria; Univ Frauenklinik Innsbruck, Innsbruck, Austria; JNSZ Megyei Hetényi Géza Kórház-Rendelőintézet, Szolnok, Hungary; ARNAS Garibaldi, Catania, Italy; Onkonet – Onkologie Ravensburg, Ravensburg, Germany; Maastricht University Medical Center, Maastricht, Netherlands; Rainier Hematology-Oncology/Northwest Medical Specialities, LLC, Tacoma, WA; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), Meldola, Italy; Novartis Pharma AG, Basel, Switzerland; Novartis Pharmaceuticals Corporation, East Hanover, NJ and Centre Léon Bérard, Lyon, France.

Body: Background: Phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathway activation is a hallmark of hormone receptor-positive (HR+) breast cancer (BC) resistant to endocrine therapy (ET). Preclinical and clinical data suggest that adding a PI3K inhibitor (PI3Ki) to ET may overcome resistance. In BELLE-2, a Phase III randomized study, buparlisib (BUP; BKM120; pan-PI3Ki) + fulvestrant (FULV) demonstrated clinical activity and manageable safety in patients (pts) with HR+, human epidermal growth factor receptor 2-negative advanced BC, with the greatest treatment effect in pts with *PIK3CA* mutation in circulating tumor DNA (ctDNA). Here, we report results from the final progression-free survival (PFS) analysis of the BELLE-3 study.

Methods: Pts (N=432) were randomized 2:1 to BUP (100mg/day) or placebo (PBO) + FULV (500mg per standard of care) and stratified by visceral disease status. Key inclusion criteria: prior aromatase inhibitor therapy; disease progression \leq 30 days from combination therapy of ET + mTOR inhibitor as last regimen. Key exclusion criteria: $>$ 1 chemotherapy regimen for advanced BC; prior PI3Ki, AKT inhibitor, or FULV; history of/active mood disorders. Primary and key secondary endpoints were PFS (local assessment; Response Evaluation Criteria In Solid Tumors v1.1) and overall survival (OS), respectively. Other secondary endpoints included: overall response rate (ORR); clinical benefit rate (CBR); efficacy by *PIK3CA* status in ctDNA (BEAMing technology); safety.

Results: BELLE-3 met its primary endpoint with a statistically significant improvement in PFS per investigator assessment in favor of BUP + FULV (BUP arm) vs PBO + FULV (PBO arm; hazard ratio [HR] 0.67; 95% confidence interval [CI]: 0.53–0.84; $p < 0.001$). Median PFS (mPFS) in the BUP vs PBO arm was 3.9 vs 1.8 months. The 6-month PFS rate was 30.6% vs 20.1%. PFS per central assessment was consistent with these findings (HR 0.57; 95% CI: 0.44–0.74; $p < 0.001$). *PIK3CA* status in ctDNA was available for 349 pts; 147 (42.1%) pts had *PIK3CA*-mutant (mut) status and 202 (57.9%) had *PIK3CA*-wildtype (wt) status. PFS improvement in the BUP vs PBO arm was greater in the *PIK3CA*-mut (mPFS 4.7 vs 1.6 months; HR 0.50; 95% CI: 0.33–0.76) than the *PIK3CA*-wt group (mPFS 3.7 vs 2.7 months; HR 0.73; 95% CI: 0.52–1.01). A similar treatment effect was seen in 321 pts with *PIK3CA* status based on PCR in tissue samples (*PIK3CA*-mut HR 0.39; 95% CI: 0.23–0.65. *PIK3CA*-wt HR 0.83; 95% CI: 0.60–1.14). ORR was 7.6% (95% CI: 4.8–11.3) vs 2.1% (95% CI: 0.4–6.0) in the BUP vs PBO arm, and CBR at 24 weeks was 24.6% (95% CI: 19.7–29.9) vs 15.4% (95% CI: 9.9–22.4). Most common ($>$ 10%; BUP vs PBO arm) Grade 3/4 AEs were increased alanine aminotransferase (21.9% vs 2.9%), increased aspartate aminotransferase (17.7% vs 2.9%), and hyperglycemia (12.2% vs 0).

Conclusions: BELLE-3 met its primary endpoint in the full population. PFS improvement in the BUP vs PBO arm was greater in pts with *PIK3CA*-mut than *PIK3CA*-wt tumors, based on ctDNA and PCR. Secondary endpoints showed improved clinical benefit with BUP + FULV vs PBO + FULV. Safety was in line with that previously seen with the combination.

Keywords: Breast cancer; PI3K inhibitor; Fulvestrant; Buparlisib.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-01

Title: SANDPIPER: Phase III study of the PI3-kinase inhibitor taselisib (GDC-0032) plus fulvestrant in patients with estrogen receptor-positive, HER2-negative locally advanced or metastatic breast cancer enriched for patients with *PIK3CA*-mutant tumors

Baselga J, Cortés J, De Laurentiis M, Dent S, Diéras V, Harbeck N, Hsu J, Jin H, Schimmoller F, Wilson TR R, Im Y-H, Jacot W, Krop IE E and Verma S. Memorial Sloan Kettering Cancer Center, Memorial Hospital, New York, NY; Vall d'Hebron Institute of Oncology (VHIO) / Ramón y Cajal University Hospital, Barcelona / Madrid, Spain; Istituto Nazionale Tumori Fondazione G. Pascale, Naples, Italy; The Ottawa Hospital Cancer Centre, Ottawa, ON, Canada; Institut Curie, Paris, France; Brustzentrum der Universität München (LMU), Munich, Germany; Genentech Inc., South San Francisco, CA; Samsung Medical Center, Seoul, Republic of Korea; Institut du Cancer de Montpellier (ICM), Montpellier, France; Dana-Farber Cancer Institute, Boston, MA and Tom Baker Cancer Centre, University of Calgary, Calgary, AB, Canada.

Body: Background: *PIK3CA* mutations frequently occur in breast cancer (BC), being present in ~40% of estrogen receptor (ER)-positive, HER2-negative breast tumors. *PIK3CA* mutations promote growth and proliferation of tumors and mediate resistance to endocrine therapies in BC. Taselisib is a potent and selective PI3-kinase (PI3K) inhibitor that displays greater selectivity for mutant PI3K α than wild-type PI3K α through a unique mechanism. In cell studies, taselisib preferentially degraded mutant compared with wild-type PI3K α , which was not seen with alpelisib and pictilisib. Taselisib has enhanced activity against *PIK3CA*-mutant BC cell lines, and clinical data include confirmed partial responses in patients with *PIK3CA*-mutant BC treated with taselisib either as a single agent or in combination with fulvestrant.

Trial design: SANDPIPER is a double-blinded, placebo-controlled, randomized, phase III study, designed to evaluate efficacy and safety of taselisib plus fulvestrant in patients with ER-positive, HER2-negative locally advanced or metastatic BC. Patients will be randomized 2:1 to receive either taselisib (4 mg daily) or placebo plus fulvestrant (500 mg intramuscular on Days 1 and 15 of Cycle 1, and on Day 1 of each subsequent 28-day cycle). Randomization will be stratified by visceral disease, endocrine sensitivity, and geographic region. The study enriches for patients with *PIK3CA*-mutant tumors who will be randomized separately from those with non-mutant tumors.

Eligibility: Postmenopausal women with ER-positive, HER2-negative, locally advanced or metastatic BC are eligible if they have disease recurrence or progression during or after aromatase inhibitor treatment. A valid cobas[®] *PIK3CA* Mutation Test result via central assessment is required prior to enrollment.

Aims: The primary efficacy endpoint is investigator-assessed progression-free survival (PFS) in patients with *PIK3CA*-mutant tumors. Additional endpoints include overall survival (OS), objective response rate (ORR), clinical benefit rate (CBR), duration of objective response, safety, pharmacokinetics, and patient-reported outcomes.

Statistical methods: The primary efficacy analysis population will include all randomized patients with *PIK3CA*-mutant tumors. Patients will be grouped according to randomized treatment arm. Median PFS and OS will be estimated using Kaplan–Meier methodology. Cox proportional-hazards models, stratified by the stratification factors, will be used to estimate the hazard ratio with 95% confidence intervals (CIs). ORR, CBR, and their 95% CIs will be estimated. Duration of objective response will be estimated using Kaplan–Meier methodology. Quality of life will be analyzed and summarized. Safety will be analyzed for all treated patients according to actual treatment received.

Accrual: Target enrollment is 600 patients. The study is open for enrollment and, as at April 2016, over 200 patients have been enrolled. Clinicaltrials.gov ID: NCT02340221.

Contact information: For more information or to refer a patient, email global.roche-genentechtrials@roche.com or call 1-888-662-6728 (USA only).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-02

Title: COLET: A multistage, phase 2 study evaluating the safety and efficacy of a doublet regimen of cobimetinib (C) in combination with paclitaxel (P) or triplet regimens of C in combination with atezolizumab (atezo) plus either P or nab-paclitaxel (nab-P) in metastatic triple-negative breast cancer (TNBC)

Miles D, Kim S-B, McNally V, Simmons B, Wongchenko M, Xu N and Brufsky A. Mount Vernon Cancer Centre, London, United Kingdom; Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; Roche Products Ltd., Welwyn Garden City, United Kingdom; Genentech, Inc., South San Francisco, CA and University of Pittsburgh, Pittsburgh, PA.

Body: Preclinical data suggest that upregulation of the MAPK pathway confers resistance to taxane chemotherapy. Mutations and gene amplifications in the MAPK pathway are present in many TNBC tumors and may contribute to taxane resistance. Preliminary data from an initial safety run-in stage of the COLET study (ClinicalTrials.gov ID, NCT02322814; EudraCT number, 2014-002230-32) suggest improvement of clinical outcomes when MEK inhibition is combined with taxane chemotherapy. Additionally, in preclinical models, MEK inhibition was shown to enhance anti-PD-L1 activity. The monoclonal antibody PD-L1 inhibitor atezo has shown promising activity in combination with nab-P in metastatic TNBC. Accordingly, the COLET protocol was amended to include the evaluation of triplet regimens combining atezo with MEK inhibition and taxane chemotherapy[SL1]. COLET is evaluating the safety and efficacy of various combinations of C as first-line treatment for metastatic or locally advanced TNBC. Key eligibility criteria include measurable disease per Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST v1.1) and left ventricular ejection fraction > institutional lower limit of normal or >50%. Neoadjuvant or adjuvant therapy is allowed if completed >6 months prior to study entry. COLET has 3 cohorts: I, II, and III. Cohort I has 2 stages: an initial safety run-in stage (n~12) followed by an expansion stage (n~90) of 1:1 randomization to C + P or placebo (PBO) + P. Patients received P 80 mg/m² on days 1, 8, and 15 and C/PBO 60 mg/day on days 3-23 of each 28-day cycle. In the expansion stage of Cohort I, randomization is stratified by prior neoadjuvant/adjuvant taxane therapy and disease-free interval from last chemotherapy dose. Cohorts II and III will evaluate the safety and efficacy of adding atezo to C + P or nab-P, respectively. Each cohort has a safety run-in stage (n~15) and an expansion stage (additional n~15); each will receive atezo 840 mg on days 1 and 15 and C 60 mg/day on days 3-23 of every 28-day cycle. Cohort II will receive P 80 mg/m² and Cohort III will receive intravenous nab-P 100 mg/m² on days 1, 8, and 15. Patients will receive treatment until disease progression or toxicity. The primary efficacy end point is investigator-assessed progression-free survival (PFS) for the expansion stage (Cohort I), and the primary PFS analysis will be performed when 60 PFS events occur across the 2 arms. This provides 77% power to detect a hazard ratio of 0.5 at a two-sided significance level of 0.05. For Cohorts II and III, the primary efficacy end point is overall response rate per RECIST v1.1; secondary end points include duration of response, PFS, and overall survival. Recruitment into the safety run-in stage of Cohort I is complete. Accrual into the randomization stage of Cohort I and the initial safety run-in stage of Cohorts II and III are ongoing. Patients from sites across North America, Europe, and the Asia-Pacific region will be enrolled.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-03

Title: Phase II Trial of the addition of pembrolizumab to letrozole and palbociclib in patients with metastatic estrogen receptor positive breast cancer who have stable disease on letrozole and palbociclib

Yuan Y, Frankel P, Synold T, Yost S, Lee P, Waisman J, Somlo G, Hurria A and Mortimer J. City of Hope, Duarte, CA.

Body: Background: The combination of palbociclib and letrozole has become the standard of care for patients with newly diagnosed estrogen receptor positive (ER+) metastatic breast cancer (MBC), with promising prolongation of progression free survival (PFS). However, nearly half of all patients achieved stable disease only after the first 6 months of therapy. Check-point inhibitor pembrolizumab was effective in ER+ MBC with a response rate of 13-17%, this study will evaluate the efficacy of adding pembrolizumab for patients with ER+ MBC who have achieved stable disease (SD) on letrozole and palbociclib.

Trial Design: This is an open-label single institutional study. Patient will receive letrozole (2.5 mg) once a day and palbociclib (125 mg, 100 mg, or 75 mg as established tolerated dose) once a day for 3 weeks on and 1 week off. Pembrolizumab will be given at 200 mg IV every 3 weeks.

Eligibility Criteria: Eligible patients must be postmenopausal women with ER+ MBC with measurable disease by RECIST1.1, ECOG performance status 0-1; must have received letrozole and palbociclib for at least 6 months, and have documented SD per RECIST 1.1. Up to 3 lines of previous systemic therapy including endocrine therapy and/or chemotherapy are allowed. Patients are excluded if they had prior treatment with anti-PD1 or anti-PD-L1 therapy, immunodeficiency; currently using systemic steroids active tuberculosis infection; major surgery within 28 days; active or untreated CNS metastases; history of interstitial lung disease; active infection requiring systemic therapy; or active cardiac disease.

Specific Aims: The primary objective is to evaluate the objective response rate (ORR). The secondary objective is to determine the safety and tolerability of pembrolizumab plus the letrozole/palbociclib combination. We will use clinical benefit rate (CBR), duration of response (DOR), PFS, and OS to test the efficacy of this novel drug combination.

Statistical Design: We will employ a three-at-risk design (modified rolling design) for the initial cohort of this Phase II study to insure the triplet is well-tolerated. This design permits only 3 patients to be at risk for DLT at any one time during the "safety lead-in". When the first 6 patients have completed the observation period and treatment with ≤ 1 DLT, the safety lead-in for the triplet will be considered successful, and accrual will proceed to a total of 18 patients. Response (CR or PR by RECIST version 1.1) in patients who have demonstrated only SD on letrozole and palbociclib can be reasonably attributed to the addition of pembrolizumab. As a result, we set the probability of a response occurring without the addition of pembrolizumab as 3% or less. With 18 patients, a true response rate of 20% would result in at least 2 responders with 90% power and a type I error of 10%. With 18 patients, the response can be estimated with a 95% CI half-width of 23%.

Target Accrual: 18.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-04

Title: SOLAR-1: A phase III study of alpelisib and fulvestrant in men and postmenopausal women with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) advanced breast cancer (BC) progressing on or after aromatase inhibitor (AI) therapy

Andre F, Kaufman B, Juric D, Ciruelos EM M, Iwata H, Mayer IA A, Rugo HS S, Conte P, Liobl S, Rubovszky G, Inoue K, Tesch H, Lu Y-S, Ryvo L, Longin A-S, Mills D, Wilke C, Germa C and Campone M. Institut Gustave Roussy, Villejuif, France; The Chaim Sheba Medical Center, Ramat-Gan, Israel; Massachusetts General Hospital, Boston, MA; Hospital Universitario 12 de Octubre, Madrid, Spain; Aichi Cancer Center Hospital, Nagoya, Japan; Vanderbilt-Ingram Cancer Center, Nashville, TN; University of California San Francisco Comprehensive Cancer Center, San Francisco, CA; University of Padova and Istituto Oncologico Veneto, Padova, Italy; GBG Forschungs GmbH, Neu-Isenburg, Germany; Országos Onkológiai Intézet, Budapest, Hungary; Saitama Cancer Center, Saitama, Japan; Onkologische Gemeinschaftspraxis, Frankfurt, Germany; National Taiwan University Hospital, Taipei City, Taiwan; Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; Novartis Pharma S.A.S., Cedex, France; Novartis Pharma AG, Basel, Switzerland; Novartis Pharmaceuticals Corporation, East Hanover, NJ and Institut de Cancérologie de l'Ouest Site Centre René Gauducheau, Saint-Herblain, France.

Body: Background: The phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathway is often dysregulated in HR+ BC and is associated with resistance to endocrine therapy (ET). Alpelisib (BYL719; PI3K α -specific inhibitor) and fulvestrant showed signs of antitumor activity in patients (pts) with estrogen receptor-positive (ER+), HER2- advanced BC (phase I), especially in *PIK3CA*-altered tumors (Janku *et al.* SABCS 2014, PD5-5).

Methods: SOLAR-1 (NCT02437318) is a phase III, randomized, double-blind study in men and postmenopausal women with HR+, HER2- advanced BC. Pts are assigned to 1 of 2 cohorts based on *PIK3CA* tumor status (mutant vs non-mutant), and randomized 1:1 to oral alpelisib/placebo (300 mg once daily) and intramuscular fulvestrant (500 mg on Day 1 and 15 of Cycle 1; Day 1 of Cycles ≥ 2 [28-day cycles]) until disease progression or discontinuation. Randomization is stratified by presence of liver and/or lung metastases and prior CDK4/6 inhibitor therapy. Key inclusion criteria: recurrence or progression on or after AI therapy, ≥ 1 measurable lesion (RECIST v1.1) or predominantly lytic bone lesion, and ECOG performance status ≤ 1 . Key exclusion criteria: symptomatic visceral disease or disease burden precluding ET, acute pancreatitis ≤ 1 year prior to screening or history of chronic pancreatitis, and prior therapy with fulvestrant, chemotherapy (except [neo]adjuvant), or PI3K/AKT/mTOR inhibitors.

The primary and key secondary endpoints are progression-free survival (PFS; RECIST v1.1; local assessment) and overall survival (OS), respectively, in the *PIK3CA*-mutant cohort. Other secondary endpoints include PFS and OS in the *PIK3CA* non-mutant cohort, PFS (Blinded Independent Central Review; RECIST v1.1), the association between PFS and baseline *PIK3CA* status in circulating tumor DNA, overall response rate, clinical benefit rate, safety, and pharmacokinetics. The primary endpoint will be analyzed by a stratified log-rank test at one-sided 2% level of significance.

Recruitment of the planned 560 pts is ongoing.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-05

Title: TRINITI-1: Ribociclib + everolimus (EVE) + exemestane (EXE) triplet combination in men or postmenopausal women with HR+, HER2– advanced breast cancer (ABC) following progression on a cyclin-dependent kinase (CDK) 4/6 inhibitor

Bardia A, Hurvitz S, Yardley DA A, Zelnak A, DeMichele A, Clark AS S, Warsi G, Small T, Tucci C and Moulder S. Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA; University of California, Los Angeles, Santa Monica, CA; Sarah Cannon Research Institute and Tennessee Oncology PLLC, Nashville, TN; Northside Hospital, Atlanta, GA; University of Pennsylvania Abramson Cancer Center, Philadelphia, PA; Novartis Pharmaceuticals Corporation, East Hanover, NJ and The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: There is extensive crosstalk between the cyclin D–CDK4/6–inhibitor of CDK4–retinoblastoma and phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathways at the G1/S cell-cycle checkpoint. Both pathways are frequently dysregulated in hormone receptor-positive (HR+) breast cancer and have been associated with endocrine therapy (ET) resistance. CDK4/6 and PI3K/mTOR inhibitors have demonstrated clinical activity in combination with ET. Although CDK4/6 inhibitors combined with ET significantly improve progression-free survival (PFS) in ABC, disease progression eventually occurs, highlighting the need for effective treatment options following doublet therapy. Ribociclib (LEE011; CDK4/6 inhibitor; 3-weeks-on/1-week-off) + EVE (mTOR inhibitor) + EXE triplet therapy has shown preliminary clinical activity in heavily pretreated HR+, human epidermal growth factor receptor 2-negative (HER2–) ABC including patients (pts) with prior exposure to CDK4/6 inhibitors, suggesting this combination may restore sensitivity to CDK4/6 inhibitor-based therapy.

Trial design and objectives: TRINITI-1 (NCT02732119) is a US-based, phase I/II, single arm, open-label study of ribociclib (continuous daily dosing) + EVE (2.5 mg/day) + EXE (25 mg/day) in men and postmenopausal women with HR+, HER2– ABC refractory to ≥ 1 line of ET. Phase I dose escalation consists of 2 ribociclib dose-level cohorts (250 and 300 mg/day), followed by a Simon Two-Stage phase II trial in pts with disease progression on prior CDK4/6 inhibitor-based therapy. No more than 3 lines of therapy for ABC, including ≤ 1 prior chemotherapy regimen, are permitted. Previous EXE treatment of >28 days for metastatic disease, prior mTOR inhibitors, and progression on >1 CDK4/6 inhibitor are prohibited. All pts in phase II must have progressed on a CDK4/6 inhibitor as the last regimen before study entry. Additional eligibility criteria include measurable disease or lytic/mixed bone lesions and Eastern Cooperative Oncology Group performance status of ≤ 1 . Exclusion criteria include visceral crisis, unstable CNS metastases, and clinically significant heart disease. Phase I primary objective: maximum tolerated dose and/or recommended phase II dose of the triplet combination. Phase II primary objective: clinical benefit rate (CBR) at 24 weeks (0.1 significance level, 80% power to test CBR $\leq 15\%$ against CBR $\geq 30\%$) with centrally-assessed PFS as a key secondary objective. Other secondary objectives include preliminary antitumor activity (phase I), safety and pharmacokinetics (phase I/II), and overall response rate, overall survival, and duration of overall response (phase II). Tumor assessments (RECIST v1.1) will be performed every 8 weeks for the first 12 months, and every 12 weeks thereafter until disease progression. Exploratory analyses include biomarkers potentially predictive of response and mechanisms of resistance.

Target accrual: Approximately 52 pts at ~ 30 sites; 3–6 pts per cohort in phase I, an initial 19 in phase II with another 20 enrolled upon demonstration of clinical benefit in ≥ 4 pts.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-06

Title: Phase III study of palbociclib (PD-0332991) in combination with endocrine therapy (exemestane or fulvestrant) versus chemotherapy (capecitabine) in hormonal receptor (HR) positive/HER2 negative metastatic breast cancer (MBC) patients with resistance to aromatase inhibitors. *“The PEARL study”* (GEICAM/2013-02)

Martín M, Kahan Z, Carrasco E, Bartlett CH H, Casas M, Gil-Gil M, Muñoz M, Ciruelos EM M, Ruiz-Borrego M, Margelí M, Antón A, Bermejo B, Morales S, Gal-Yam E, Koehler M, García-Sáenz JA A, de la Haba J, Chacón I and Zielinski C. Instituto de Investigación Sanitaria Gregorio Marañón-Universidad Complutense de Madrid, Madrid, Spain; Onkoterápiás Klinika, Szeged, Hungary; GEICAM, Madrid, San Sebastián de los Reyes, Madrid, Spain; Pfizer Inc., NYC; Institut Catalá d'Oncologia (ICO), L'Hospitalet de Llobregat, Barcelona, Spain; Hospital Universitari Clinic de Barcelona, Barcelona, Spain; Hospital Universitario 12 de Octubre, Madrid, Spain; Hospital Universitario Virgen del Rocío, Sevilla, Spain; Hospital Universitari Germans Trias i Pujol, Badalona, Spain; Hospital Universitario Miguel Servet, Zaragoza, Spain; Hospital Clínico Universitario de Valencia, Valencia, Spain; Hospital Universitario Aranau de Vilanova, Lleida, Spain; Sheba Medical Center, Tel Hashomer, Israel; Hospital Universitario Clínico San Carlos, Madrid, Spain; Hospital Universitario Reina Sofía, Córdoba, Spain; Complejo Hospitalario Virgen de la Salud, Toledo, Spain and Univ. Klinik für Innere Medizin I, Währinger Gürtel, Vienna, Austria.

Body: Background: Endocrine therapy (ET) is the cornerstone treatment for HR-positive, HER2-negative breast cancer patients. However, endocrine resistance is a major clinical challenge. Treatment options at recurrence/progression to AIs include sequential endocrine-based therapies in monotherapy or in combination with a targeted agent or chemotherapy. Preclinical data suggest that ER+/HER2- BC is dependent on cyclin-dependent kinases 4/6 (CDK4/6) function; the inhibition of this target may be effective in overcome endocrine resistance. Palbociclib (P) is an oral novel CDK4/6 inhibitor that is synergistic with ET in preclinical and clinical studies. Initially this study was designed to demonstrate the clinical benefit of P plus exemestane (E) vs capecitabine (C) (cohort1). Recent studies revealed that the acquisition of *ESR1* mutations is a major mechanism of resistance to the treatment of AIs in the metastatic setting. Retrospective analyses show that patients with *ESR1* mutation derived no benefit from sequential AI monotherapy. In contrast, preclinical and clinical data indicated that SERD, fulvestrant (F), is active in *ESR1* mutant tumors. Hence the choice of endocrine partner with P is particularly important to create optimal synergy in endocrine resistance setting. The current design added cohort 2 of P plus F vs C. The overall study goal is to prospectively answer the question of optimal endocrine partner with P and its efficacy/safety over chemotherapy in endocrine resistant setting.

Trial Design: This is an international (5 countries) randomized phase III study with 2 cohorts, patients will be randomized 1:1 to ET (cohort 1: E 25 mg daily, cohort 2: F 500mg days 1 and 15 cycle 1 and then day 1 every 4 weeks) plus P (125 mg daily x3 weeks every 4 weeks) vs. C (1,250 mg/m² twice daily x2 weeks every 3 weeks). Postmenopausal patients with HR+/HER2- MBC are eligible if resistant to previous AI (letrozole or anastrozole in cohort 1 or any AI in cohort 2) defined as: recurrence while on or within 12 months after the end of adjuvant treatment or progression while on or within 1 month after the end of treatment for MBC. Previous chemotherapy is permitted either in the (neo)adjuvant setting and/or as first line for MBC. Patients must have *measurable disease according to RECIST 1.1 or bone lesions, lytic or mixed, in the absence of measurable disease*. The primary objectives are to demonstrate that P plus F is superior to C and that P plus ET is superior to C in women whose tumor had estrogen receptor *ESR1* wild type in terms of Progression-Free Survival (PFS); secondary objectives are PFS regardless the *ESR1* mutational status, overall survival, response rate, clinical benefit rate, response duration, safety, quality of life and biomarkers. The study will recruit approximately 302 patients in cohort 1 and 300 patients in cohort 2. The study started recruitment in March 2014; 290 patients have been included so far (289 cohort1 + 1 cohort 2). Analysis of primary endpoint is planned in Sep2019 (**ClinTrials.gov reference NCT02028507**).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-07

Title: A phase 2 study of abemaciclib plus pembrolizumab for patients with hormone receptor positive (HR+), HER2 negative (HER2-) metastatic breast cancer (MBC)

Rugo H, Tolaney S, Dickler M, Kabos P, Ho C-L, Wildiers H, Jerusalem G, Alés-Martínez JE, Hossain A, Johnston E and Gianni L. University of California San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; Dana-Farber Cancer Institute, Boston, MA; Memorial Sloan-Kettering Cancer Center, New York, NY; University of Colorado Anschutz Medical Campus, Aurora, CO; Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; Universitair Ziekenhuis Leuven (Gasthuisbert), Leuven, Belgium; Centre Hospitalier Universitaire du Sart Tilman, Liège, Belgium; Hospital Nuestra Señora de Sonsoles, Complejo Hospitalario de Ávila – Oncology, Ávila, Spain; Eli Lilly and Company, Indianapolis, IN and IRCCS Ospedale, Milano, Italy.

Body: Background:

Abemaciclib is a small molecule inhibitor of both cyclin-dependent kinase (CDK) 4 and CDK6 administered orally twice daily on a continuous schedule. In I3Y-MC-JPBA, a phase I study, abemaciclib demonstrated acceptable safety, tolerability, and single-agent activity as monotherapy in different tumor types, including HR+ MBC (Patnaik A, et al. *Cancer Discov* 2016;6:1–14). In MONARCH 1, a phase 2 study, abemaciclib demonstrated a 19.7% objective response rate (ORR) with a median duration of response (DoR) of 8.6 months, median progression-free survival (PFS) of 6.0 months, and clinical benefit rate (CBR) of 42.4% in patients with HR+, HER2- MBC whose disease progressed on or after endocrine therapy and chemotherapy (Dickler, M. et al. American Society of Clinical Oncology (ASCO), abstract #510 (2016)). Pembrolizumab is a humanized monoclonal antibody against the programmed death receptor-1 (PD-1) protein that has shown preliminary efficacy in single-arm monotherapy trials in ER+/HER2- advanced breast cancer.

Trial design:

This open-label, phase 2 study will evaluate the safety and preliminary efficacy of abemaciclib 150 mg given orally every 12 hours on days 1-21 of a 21-day cycle in combination with intravenous pembrolizumab 200 mg on day 1 of a 21-day cycle in approximately 75 patients with stage IV non-small cell lung cancer or HR+, HER2- MBC (ClinicalTrials.gov NCT02779751). The study will include 3 disease-specific cohorts, each with approximately 25 patients. Only the HR+, HER2- MBC cohort will be presented here.

Eligibility criteria:

Eligible patients for the MBC cohort include women with confirmed HR+, HER2- MBC who have completed at least 1 but no more than 2 prior chemotherapy regimens in the metastatic setting; will provide tumor tissue prior to and after treatment (cycle 3, day 1); have measurable disease (RECIST v.1.1), adequate organ function, an ECOG performance status ≤ 1 , and a life expectancy ≥ 12 weeks; are ≥ 18 yrs of age and able to swallow oral medications; and have not received treatment with any CDK 4 and 6 inhibitors or PD-1 or PD-L1 inhibitors.

Specific aims:

The primary objective is to characterize the safety profile of the combination of abemaciclib and pembrolizumab. Key secondary objectives include ORR, DoR, disease control rate (DCR), PFS, overall survival (OS), and characterization of pharmacokinetics.

Statistical methods:

The safety population includes patients who received at least one dose of study drug. ORR, DoR, DCR, and PFS analyses will be evaluated according to RECIST v.1.1 and irRECIST for disease progression. Time-to-event variables, such as DoR, PFS, and OS, will be estimated by Kaplan-Meier methodology. An interim analysis of safety and preliminary efficacy may occur for each cohort after all patients have completed (or discontinued from) approximately 24 weeks of treatment. The final analysis of OS will occur based on data collected for approximately 12 months after the last patient receives treatment.

Target accrual:

Approximately 75 patients are planned for the trial; 25 patients will comprise the MBC cohort.

Contact information: 1-877-CTLILLY (1-877-285-4559).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-08

Title: Phase 2 study of abemaciclib plus tamoxifen or abemaciclib alone in women with previously treated hormone receptor-positive (HR+), HER2- metastatic breast cancer

Diéras V, Hamilton E, Johnston EL L, Forrester T and Martín M. Institut Curie Paris, Paris, France; Sarah Cannon Research Institute, Nashville, TN; Eli Lilly and Company, Indianapolis, IN and Hospital General Universitario Gregorio Marañón, Madrid, Spain.

Body: Background:

Abemaciclib, an oral drug administered twice daily on a continuous schedule, is a specific inhibitor of both CDK4 and CDK6. In MONARCH 1, abemaciclib demonstrated evidence of single-agent activity and an acceptable safety profile in patients with heavily pretreated HR+, HER2- metastatic breast cancer (MBC) whose disease progressed on or after endocrine therapy and chemotherapy; with a confirmed objective response rate of 19.7% and a median duration of response of 8.6 months (Dickler, M. et al. American Society of Clinical Oncology (ASCO), abstr #510 (2016)). This Phase 2 study I3Y-MC-JPCG will evaluate the potential to enhance the risk/benefit profile of abemaciclib in women with previously treated HR+, HER2- MBC, either administered in combination with endocrine therapy or as monotherapy (at the maximum recommended single-agent dose with primary prophylactic antidiarrheal medication or at a lower dose of abemaciclib monotherapy). Such intervention with prophylactic loperamide may increase dose intensity and further optimize the clinical benefit for patients.

Trial design:

Study JPCG (NCT02747004) is a Phase 2 multicenter, randomized, open-label trial with 3 separate treatment arms in patients with HR+, HER2- MBC who have progressed on or after prior endocrine therapy and have received prior treatment with chemotherapy. Patients will be stratified based on presence of liver metastases and prior use of tamoxifen in the advanced/metastatic setting and will be randomized in a 1:1:1 ratio to receive abemaciclib 150 mg Q12H plus tamoxifen 20 mg every day (Arm A) or abemaciclib 150 mg Q12H monotherapy (Arm B); or abemaciclib 200 mg Q12H monotherapy plus primary prophylactic loperamide (Arm C).

Eligibility criteria:

Eligible patients include women with HR+, HER2- MBC with evidence of relapse or disease progression following endocrine therapy who have received prior treatment with at least 2 chemotherapy regimens, of which at least 1 but not more than 2 regimens have been administered in the metastatic setting. Patients are required to have measurable disease, adequate organ function, an ECOG PS of ≤ 1 , and no prior treatment with any CDK4 and CDK6 inhibitor.

Specific aims:

The primary objective of JPCG is to evaluate the progression-free survival per RECIST v1.1, of abemaciclib 150 mg Q12H plus tamoxifen 20 mg QD, abemaciclib 150 mg Q12H monotherapy, and abemaciclib 200 mg Q12H plus prophylactic loperamide in women with HR+, HER2- MBC who have progressed on or after prior endocrine therapy and received prior chemotherapy. Secondary objectives are to evaluate response rates, overall survival, safety, pharmacokinetics, and quality of life. Exploratory objectives include evaluation of the associations between biomarkers relevant to abemaciclib and clinical outcomes.

Statistical methods:

Assuming a hazard ratio of 0.667, the study yields approximately 80% statistical power to detect superiority of Arm A over Arm C at a 1-sided alpha level of 0.10 using a log-rank test. Arms B and C will be compared using an informal non-inferiority rule.

Target accrual:

Approximately 225 patients.

Contact information:

For further information, please contact 1-877-CTLILLY (1-877-285-4559).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-09

Title: PALINA: A phase II safety study of palbociclib in combination with letrozole in African American women with hormone receptor positive HER2 negative advanced breast cancer

Lynce F, Shajahan-Haq A, Cai L, Graham D, Gallagher C, Mohebtash M, Kamugisha L, Novielli N, Castle J, Forero A and Isaacs C. Lombardi Comprehensive Cancer Center, MedStar Georgetown University Hospital, Washington, DC; Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC; Hackensack University Medical Center, New Jersey, NJ; MedStar Washington Hospital Center, Washington, DC; MedStar Union Memorial Hospital, Baltimore; MedStar Good Samaritan Hospital, Baltimore and University of Alabama, Birmingham, Al.

Body: Background: Insufficient data exist to describe the hematological safety of palbociclib in African American women (AAW) who are known to have a high incidence of benign ethnic neutropenia (BEN). The studies that led to the FDA approval of palbociclib (PALOMA 1 and 3) only included participants with baseline absolute neutrophil count (ANC) of $\geq 1500/\text{mm}^3$. The standard lower limit of ANC of $1500/\text{mm}^3$ for initiation of treatment in those with BEN has been previously challenged. In this current study, we propose to lower the ANC cutoff for enrollment to $1000/\text{mm}^3$.

Trial design: PALINA is a phase II study evaluating the hematological safety of palbociclib with letrozole in 35 AAW with hormone receptor (HR) positive HER2 negative advanced breast cancer and $\text{ANC} \geq 1000/\text{mm}^3$. Patients enrolled will receive palbociclib 125mg daily for 21 days followed by 7 days off and letrozole 2.5mg daily. For patients enrolled with baseline ANC between $1000\text{-}1499/\text{mm}^3$, initial dose of palbociclib will be 100mg daily for 21 days followed by 7 days off. Presence of Duffy Null Polymorphism (SNP rs2814778) as a predictive marker for neutrophil count will be assessed at baseline. Metabolite and exosomal signature (proteins and RNA) of drug resistance will be evaluated at different time points.

Main eligibility criteria: Self-identified Black, African or AAW of ≥ 18 years of age with proven diagnosis of advanced HR-positive, HER2-negative breast cancer; ECOG performance status 0-2; $\text{ANC} \geq 1,000/\text{mm}^3$ and no prior receipt of CDK4/6 inhibitors.

Specific aims: The primary endpoint is the proportion of patients who complete planned oncologic therapy without the development of a hematological event defined as episodes of febrile neutropenia and treatment discontinuation due to neutropenia. Additional endpoints include: number of patients who required dose delays or dose reductions in palbociclib attributed to neutropenia; rate of grade 3/4 neutropenia; clinical benefit rate at 24 weeks; correlations between metabolite and exosomal signature with disease response; correlations between baseline ANC prior to cancer diagnosis and the Duffy Null polymorphism with hematological safety.

Statistical methods: The study is designed to assess the rate of completion of planned therapy in the absence of a hematological event defined as episodes of febrile neutropenia and treatment discontinuation due to neutropenia. Simon's two-stage design with a maximum of 35 patients is used. The null hypothesis that the true completion rate is 60% will be tested against a one-sided alternative. This design yields a type I error rate of 0.05 and power of 80% when the true completion rate is 80%.

Present accrual and target accrual: The Institutional Review Board at Georgetown University Medical Center (GUMC) has approved the study. Enrollment of the first patient is expected in July 2016 with a total of 35 patients planned to be recruited. The recruitment sites are MedStar Georgetown University Hospital and other hospitals of the Georgetown MedStar Cancer Network, Hackensack University Medical Center and University of Alabama at Birmingham. This trial is funded by an ASPIRE Breast Cancer Research Award from Pfizer.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-10

Title: Pilot study of carboplatin, nab-paclitaxel and pembrolizumab for metastatic triple-negative breast cancer (ongoing clinical trial)

Baar J, Abraham J, Silverman P, Budd GT, Vinayak S, Varadan V, Moore H, Montero A and Fu P. Seidman Cancer Center, Cleveland, OH; Taussig Cancer Center, Cleveland, OH and Case Comprehensive Cancer Center, Cleveland, OH.

Body: **BACKGROUND.** Triple-negative breast cancer (TNBC) is associated with an aggressive phenotype and decreased survival. TNBC is characterized by tumor-infiltrating lymphocytes (TIL) which predict for a better prognosis and likely reflect immune recognition of tumor-associated antigens by TIL. However, potent immune suppressive signals exist in the tumor microenvironment such as those mediated by PD-1 with its ligand, PD-L1. Therefore, to test the validity of decreasing PD-1/PD-L1-mediated immune suppression, a Phase Ib study of single-agent pembrolizumab in 32 patients with advanced TNBC showed a partial response of 16.1% and stable disease of 9.7%, thereby attesting to the effectiveness of single-agent pembrolizumab in these patients. Other studies have demonstrated that cytotoxic chemotherapy favorably modulates immunity against cancer and there is therefore a strong rationale to combine chemotherapy with an immune modulator such as pembrolizumab for the treatment of mTNBC.

TRIAL DESIGN. This is an investigator-initiated, industry-sponsored (Merck) pilot study of carboplatin (C), nab-paclitaxel (N) and pembrolizumab (P) in 30 patients with metastatic (m) TNBC. Eligible patients will receive 3 cycles of CNP, with each cycle consisting of C (AUC 6 on days 1 of a 21-day cycle), N (100 mg/m² IV on days 1, 8 and 15 of a 21-day cycle), and P (200 mg IV on day 15 of each cycle). After completion of 3 cycles CNP, patients with responding or stable disease by RECIST 1.1 criteria will be eligible for additional cycle(s) of CNP.

ELIGIBILITY CRITERIA. Patients must have radiologically measurable mTNBC, an ECOG performance status of 0-1, must not have received more than 2 prior therapies for this disease, and must be willing to undergo a preliminary biopsy of a metastatic focus for research purposes. A second post-treatment biopsy will be encouraged but will not be mandated.

SPECIFIC AIMS. The primary objective is to determine overall response rate (ORR) in patients treated with CNP. The secondary objectives are to determine progression-free survival (PFS) and safety/tolerability of CNP. Correlative objectives include the identification of pathologic and genomic correlates of response to CNP.

STATISTICAL METHODS. Clinical response will be scored using RECIST 1.1 criteria. Under the proposed treatment, the expected clinical response is about 35%. With the precision of the 2-sided 95% confidence interval for the response rate set to 0.17 (the distance to the expected response rate of 35%), the sample size required for the study is 30 patients. The true response rate of therapy will be estimated based on the number of responses using a binomial distribution and its confidence intervals will be estimated using Wilson's method. The Kaplan-Meier method will be used to estimate PFS. Factors including pathologic and genomic correlates that predict survival outcomes will be identified by Cox model or extensions of the Cox model.

TARGET ACCRUAL. We plan to enroll 30 patients over 2 years, with the first patient expected to be enrolled in September 2016.

CONTACT INFORMATION. Joseph Baar, MD, PhD. Seidman Cancer Center of University Hospitals Case Medical Center.
E-mail: joseph.baar@uhhospitals.org.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-11

Title: Phase II of POSEIDON: A phase Ib / randomized phase II trial of tamoxifen plus taselisib or placebo in hormone receptor positive, HER2 negative, metastatic breast cancer patients with prior exposure to endocrine treatment

Oliveira M, Baird RD D, van Rossum AGJ G J, Beelen K, Garcia-Corbacho J, Mandjes IAM A M, Vallier AL, van Werkhoven E, Garrigós L, Kumar S, van Tinteren H, Muñoz S, Linossi C, Rosing H, Miquel JM, Schrier M, de Vries Schultink A, Saura C, Gallagher WM M, Bernards R, Tabernero J, Cortés J, Caldas C and Linn SC C. Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; Cambridge Cancer Centre, Cambridge, United Kingdom; Netherlands Cancer Institute, Amsterdam, Netherlands; Hospital Clinic, Barcelona, Spain; UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Dublin, Ireland and Hospital Ramón y Cajal, Madrid, Spain.

Body: Background: The combination of PI3K-AKT-mTOR pathway inhibitors with endocrine therapy can improve clinical outcomes of hormone receptor positive (HR+) metastatic breast cancer (MBC) patients. Taselisib is a potent and selective PI3K inhibitor, with greater selectivity against mutant (MUT) PI3K α isoforms than wild-type (WT) via a unique mechanism. Phase Ib data of POSEIDON with Taselisib + tamoxifen (TAM) demonstrated encouraging activity in patients with heavily pre-treated MBC, with an acceptable toxicity profile (Baird et al, ASCO 2016). The recommended phase II dose (RP2D) was Taselisib 4mg plus TAM 20mg, both administered on a daily continuous schedule. ctDNA monitoring may have value in drug development by (1) assessing predictive biomarkers to therapy, (2) providing an early indication of treatment response, and (3) shedding light on potential mechanisms of acquired drug resistance. In some patients included in phase Ib of POSEIDON, tumor response was preceded by a corresponding early change in plasma PIK3CA ctDNA levels. **Methods:** The phase II portion of the POSEIDON trial is a two-arm, randomized, double blind study of Taselisib plus TAM versus placebo (PLA) plus TAM in pre- and postmenopausal women with HR+/HER2- MBC. In the first part of the Phase II, 180 patients will be randomized (1:1) to receive continuous TAM with either Taselisib at the RP2D or PLA until disease progression, unacceptable toxicity or patient / physician decision. Crossover is allowed upon progressive disease in those patients receiving PLA plus TAM, after collection of tumor and blood samples for exploratory biomarker analysis. Stratification is based on menopausal status, histology [lobular breast cancer (LBC) vs. ductal/others], PIK3CA mutation (WT vs. exon 9 vs. exon 20), prior everolimus, timing of recurrence/progression after prior endocrine therapy, number of prior chemotherapy (CT) lines, and treatment center. After recruiting the initial 180 patients, trial will focus in LBC, until a total number of 110 patients with LBC are enrolled. Other key eligibility criteria include presence of measurable or evaluable disease (RECIST 1.1), prior progression to endocrine treatment, maximum of 5 prior CT lines in the metastatic setting, absence of diabetes under medical treatment, and absence of chronic inflammatory bowel disease. Primary endpoint is investigator-assessed PFS. Key secondary endpoints are PFS in LBC, objective response rate, clinical benefit rate, safety, and exploratory biomarker analysis (including ctDNA). The study has a 90% power at a two-sided log-rank test significance level of 0.2 to detect an HR of 0.64, which corresponds to an increase in median PFS from 4.5 months in the PLA plus TAM arm to 7 months in the Taselisib plus TAM arm. Enrollment to POSEIDON Phase II started in June 2016 (Clinicaltrials.gov NCT02285179).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-12

Title: ENCORE 602: A randomized, placebo-controlled, double-blind, multicenter phase 2 study (with a phase 1b lead-in) of atezolizumab with or without entinostat in patients with advanced triple negative breast cancer (aTNBC)

Forero A, Stroyakovskiy D, Cha E, Cruickshank S, Hasapidis J, Meyers ML L and Slamon DJ J. University of Alabama at Birmingham, Birmingham, AL; Moscow City Oncology Hospital N62, Moscow, Russian Federation; Genentech, Inc., South San Francisco, CA; Syndax Pharmaceuticals, Inc., Waltham, MA and University of California Los Angeles, Los Angeles, CA.

Body: Background: Atezolizumab, a humanized anti-PDL1 antibody, has shown encouraging single agent activity in triple negative breast cancer. Entinostat is an oral, class I selective histone deacetylase (HDAC) inhibitor. In animal models, entinostat has been shown to selectively reduce immunosuppressive myeloid derived suppressor cells (MDSCs) and regulatory T cells (Tregs), enhancing response to immune checkpoint blockade. It is hypothesized that entinostat in combination with atezolizumab will show improved efficacy compared to atezolizumab alone.

Trial Design: ENCORE 602 is a Phase 1b/2 study evaluating the combination of entinostat plus atezolizumab in patients with aTNBC. The study has 2 phases: an open-label Dose Determination Phase (Phase 1b) followed by Phase 2. The objective of the Dose Determination Phase is to establish the recommended Phase 2 dose (RP2D) of weekly entinostat when given in combination with atezolizumab 1200 mg every 3 weeks. Phase 2 will evaluate the efficacy and safety of entinostat at the RP2D with atezolizumab in patients with aTNBC in a randomized (1:1), double-blind, placebo-controlled setting. The randomization will be stratified by geographic location (US vs ex-US).

Key Eligibility Criteria: Eligible patients will have 1) histologically- or cytologically-confirmed triple negative breast carcinoma that is either metastatic (stage IV of the TNM classification) or locally recurrent and not amenable to local curative treatment, 2) measurable disease based on imaging studies within 28 days before the first dose of study drug, and 3) received 1-2 prior lines of systemic therapy for locally recurrent and/or metastatic disease. Previous treatment with a PD-1/PD-L1-blocking antibody or a HDAC inhibitor is not permitted.

Specific Aims: In Phase 2, the primary endpoint is progression free survival (PFS), as assessed by the investigators using RECIST 1.1. Secondary endpoints include PFS by immune response RECIST (irRECIST), overall response rate, clinical benefit rate, overall survival, safety, and duration and time to response for those patients achieving a complete or partial response. Exploratory endpoints include PK, protein lysine acetylation, and immune correlates.

Statistical Methods: The primary analysis of PFS will be performed using a stratified log-rank test. Estimation of the hazard ratio for treatment effect will be determined using a stratified Cox proportional hazards model. 60 PFS events are estimated to provide 80% power to detect the targeted improvement in PFS with one-sided significance level of 0.1. An independent data safety monitoring board will meet at regular intervals to oversee trial conduct and patient safety.

Accrual: Up to 88 evaluable patients are anticipated if the study completes all phases of evaluation (6-18 patients in Phase 1b, 70 patients in Phase 2). The study was activated in May 2016 (NCT02708680).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-13

Title: A phase 3, open-label, randomized, 2-arm international study of the oral dual PARP inhibitor talazoparib in germline *BRCA* mutation subjects with locally advanced and/or metastatic breast cancer (EMBRACA)

Litton J, Ettl J, Hurvitz SA A, Mina LA A, Rugo HS S, Lee K-H, Yerushalmi R, Woodward N, Goncalves A, Moreno F, Roche H, Im Y-H, Martin M, Bhattacharya S, Peterson A, Hannah A, Eiermann W and Blum J. MD Anderson Cancer Center, Houston, TX; Technische Universität München, Munich, Germany; University of California, Los Angeles, Los Angeles, CA; Indiana University School of Medicine, Indianapolis, IN; UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; Seoul National University Hospital, Seoul, Korea; Rabin Medical Center, Beilinson Hospital, Petah Tikva, Israel; Mater Cancer Care Centre-Mater Health Services, South Brisbane, Australia; Institut Paoli-Calmettes, Marseille, France; Hospital Clínico San Carlos, Madrid, Spain; Institut Universitaire du Cancer Toulouse, Toulouse, France; Samsung Medical Center, Seoul, Korea; Hospital General Universitario Gregorio Marañón, Madrid, Spain; Medivation, Inc., San Francisco, CA; Interdisziplinäres Onkologisches Zentrum Muenchen, Munich, Germany and Texas Oncology-Baylor Charles A. Sammons Cancer Center, Dallas, TX.

Body: Background: Cancer cells with deleterious mutations in breast cancer susceptibility genes 1 and 2 (*BRCA1/2*) are deficient in the DNA double-strand break repair mechanism, rendering them highly dependent on the single-strand break repair pathway, regulated by poly(ADP-ribose) polymerase (PARP). Inhibition of PARP results in synthetic lethality in cells with a *BRCA1/2* mutation because of accumulation of irreparable DNA damage; PARP inhibitors have the potential to be selectively toxic for *BRCA*-mutated cells. In addition to catalytic inhibition, it has been shown that some PARP inhibitors induce PARP trapping at sites of DNA damage. The capacity to trap PARP-DNA complexes varies widely across different PARP inhibitors and is not correlated with PARP catalytic inhibition. Preclinical models have shown trapping PARP on DNA is more potent at inducing cancer cell death than enzymatic inhibition of PARP alone. Talazoparib is a dual-mechanism PARP inhibitor that both inhibits the PARP enzyme and effectively traps PARP on DNA, preventing DNA damage repair and resulting in cell death in *BRCA1/2*-mutated cells. In preclinical studies, talazoparib at nanomolar concentrations showed the highest efficiency at trapping PARP-DNA complexes relative to other PARP inhibitors. In a previous phase 1/2 clinical study, talazoparib as monotherapy (1 mg once daily) resulted in a 50% response rate and an 86% clinical benefit rate at 24 weeks in 14 patients with a germline *BRCA1/2* mutation and advanced breast cancer (aBC).

Methods: This open-label, randomized, 2-arm, international phase 3 trial (EMBRACA) compares the efficacy and safety of talazoparib with protocol-specific physician's choice (capecitabine, eribulin, gemcitabine or vinorelbine) in patients with aBC. The primary objective is progression-free survival by central imaging. Secondary objectives are objective response rate, overall survival, safety and pharmacokinetics of talazoparib. Exploratory objectives include health-related quality of life measurements and biomarker research in blood and tumor samples that may permit characterization of mechanisms involved in tumor sensitivity and resistance to talazoparib. Key patient eligibility criteria include aged ≥ 18 years with histologically/cytologically confirmed breast cancer; locally advanced and/or metastatic disease appropriate for systemic single-agent cytotoxic chemotherapy; deleterious or pathogenic germline *BRCA1/2* mutations by central laboratory; ≤ 3 prior cytotoxic chemotherapy regimens for advanced disease (prior platinum is allowed provided patients did not relapse within 6 months in the adjuvant setting or did not progress on platinum therapy); prior treatment with a taxane and/or anthracycline unless medically contraindicated; and ECOG performance status ≤ 2 . Patients (N=429) will be randomized 2:1 to receive either talazoparib capsules (1 mg/day, 21-day cycles) or physician's choice treatment. This trial is currently enrolling patients from the USA, Europe, Israel, Ukraine, Russia, Korea, Australia, Taiwan and Brazil (NCT01945775). This study is funded by Medivation, Inc.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-14

Title: Triple-negative first-line study: Neoadjuvant trial of nab-paclitaxel and atezolizumab, a PD-L1 inhibitor, in patients with triple negative breast cancer (TNBC) (NCT02530489)

Litton JK K, Moulder S, Helgason T, Clayborn AR R, Rauch GM M, Gilcrease M, Adrada BE E, Huo L, Hess KR R, Symmans WF F, Thompson A, Tripathy D and Mittendorf EA A. The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: BACKGROUND: TNBC has an especially poor prognosis in patients (pts) whose tumor does not respond to anthracycline and taxane-based chemotherapy. Approximately 50% will have chemo-insensitive disease (CID) resulting in extensive residual disease at the time of surgery. 40-80% of these pts will recur \leq 3 years. Recently developed molecular profiling techniques to identify TNBC subsets detect distinct molecular hallmarks. We designed a clinical trial to identify and characterize CID (**ARTEMIS: A Randomized, TNBC Enrolling trial to confirm Molecular profiling Improves Survival**). Treatment naïve pts with localized TNBC undergo a pretreatment biopsy followed by anthracycline-based chemotherapy (AC). During AC the molecular profile is determined; these results along with the response assessment (clinical exam/diagnostic imaging) will identify CID and guide the second phase of neoadjuvant chemotherapy. Tumor-infiltrating lymphocytes (TIL) have been identified as having prognostic and predictive significance in TNBC pts leading to higher pCR rates post NACT. However, the tumor microenvironment also contains regulatory T cells and myeloid-derived suppressor cells that are immunosuppressive. Programmed death ligand 1 (PD-L1) is expressed in 20% TNBC. Targeting this may lead to a more durable response as compared to chemotherapy alone.

PRIMARY OBJECTIVE: Evaluate the rate of pathologic complete response (pCR)/RCB-0 + residual cancer burden (RCB)-I responses in TNBC pts, determined to have CID after anthracycline-based chemotherapy, then treat with atezolizumab + nab-paclitaxel preoperatively.

TRIAL DESIGN AND STATISTICAL METHODS: Pts deemed to have CID on the ARTEMIS trial can enter this non-randomized phase II study. Pts without response to their initial chemotherapy cycles have a low likelihood (5%) of achieving pCR with additional cycles of chemotherapy. It would be clinically meaningful for pCR to improve to 20%. Counting pCR (RCB-0) or RCB-I as response given similar survival outcomes, a two-stage Gehan-type design will be employed with 14 pts in the first stage. If at least one pt responds, 23 more will be added. This design has a 49% chance of terminating after the first stage if the true response rate is 0.05, 23% chance if the true rate is 0.10, 10% if the true rate is 0.15 and 4% if the true rate is 0.20. If accrual continues to the second stage, the 95% confidence interval for a 0.20 response rate will extend from 0.10 to 0.35.

BRIEF ELIGIBILITY CRITERIA: *Inclusion:* localized TNBC enrolled onto ARTEMIS and determined to have CID at the time of response assessment after anthracycline chemotherapy, adequate organ, bone marrow and cardiac parameters. *Exclusion:* prior immunotherapy, IBC, history of autoimmune disease, HIV, Hep-B, Hep-C, active tuberculosis, pregnant.

CORRELATIVE SCIENCE: Evaluate the presence and phenotype of TIL and other immune cell populations in tumor tissue pre/post treatment; determine changes in expression of co-stimulatory and co-inhibitory molecules on tumor cells and immune cells in the microenvironment; evaluate the immune repertoire and cytokine responses in serially collected peripheral blood mononuclear cells and serum respectively.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-15

Title: PARTNER - Randomised, phase II/III trial to evaluate the safety and efficacy of the addition of olaparib to platinum-based neoadjuvant chemotherapy in triple negative and/or germline BRCA mutated breast cancer patients

Abraham JE E, Vallier A-L, Qian W, Grybowicz L, Thomas S, Mahmud S, Harvey C, McAdam K, Hughes-Davies L, Roylance R, Copson E, Brown J, Provenzano E, Tischkowitz M and Earl HM M. University of Cambridge, Cambridge, Cambridgeshire, United Kingdom; Cambridge University Hospitals NHS Foundation Trust, Cambridge, Cambridgeshire, United Kingdom; University College London, London, United Kingdom; University of Southampton, Southampton, Hampshire, United Kingdom and Royal Marsden Hospital, London, United Kingdom.

Body: Background: Triple Negative Breast Cancers (TNBC) are a biologically diverse and aggressive sub-group. Early effective treatment can lead to cure. Current standard treatment is systemic chemotherapy either pre-/post-definitive surgery. No specific targeted therapies are available for TNBC. There are phenotypic and molecular similarities between germline BRCA (gBRCA) breast cancer and TNBC. In TNBC 10%-20% harbour gBRCA mutations. In gBRCA patients, and potentially other homologous recombination deficiencies, these already compromised pathways allow drugs called PARP inhibitors (olaparib) to work particularly effectively.

Aims: To establish if the addition of olaparib to neoadjuvant platinum-based chemotherapy for TNBC and/or gBRCA breast cancer is safe and improves efficacy.

Trial design: 3-stage open label randomised phase II/III trial of neoadjuvant olaparib +/- platinum containing chemotherapy followed by clinicians' choice of anthracycline regimen. Stage 1 and 2, patients are randomised (1:1:1) to either control (3 weekly carboplatin AUC5/weekly paclitaxel 80mg/m² chemotherapy - 4 cycles) or one of two research arms which uses the same chemotherapy regimen but with two different schedules of olaparib 150mg BD). Stage 3: patients are randomised (1:1) to either control arm or to the research arm selected in stage 2.

Primary outcome measures:

Stage 1: safety of the addition of olaparib to chemotherapy. Prophylactic G-CSF is mandatory.

Stage 2: pathological complete response (pCR) in each of the two research arms. At the end of stage 2, one of the research arms will be dropped.

Stage 3: pCR at surgery after neoadjuvant treatment. pCR - defined as no residual invasive carcinoma within the breast (ductal carcinoma in situ permitted) AND no evidence of metastatic disease within the lymph nodes.

Eligibility:

- Aged 16 to 70.
- Written informed consent.
- Histologically confirmed invasive breast cancer.
- Clinical stage T1-4 N0-2 (tumour or metastatic node diameter>10mm)
- Confirmed ER-negative and HER2-negative or gBRCA mutation positive, irrespective of hormone status.
- Performance Status 0-1

Statistical Methods: Stage 1, Safety: both research arms combined. Stage 2, Schedule selection criteria: pCR rate and completion rate of olaparib protocol treatment. It is a "pick-the winner" design with 53 patients in each research arm. This allows a 90% power, 5% one-sided significance level to test null hypothesis of pCR ≤35% versus an alternative hypothesis of pCR ≥55% in each of the research arms.

Stage 3, Efficacy: anticipated pCR ~45-55% for all trial patients and ~50-60% for gBRCA patients. The trial is powered to detect an absolute improvement of 15% (all patients) and 20% (gBRCA patients) by adding olaparib to chemotherapy (enriched design). TNBC patient recruitment will be capped, to ensure the required number of gBRCA patients are enrolled. Enrichment design is applied with the overall significance level $0.05(\alpha)=0.025(\alpha_{all})+0.025(\alpha_{gBRCA})$ and 80% power.

Present accrual: 1 [Trial opened: 23rd May 2016]

Target accrual: 527 (TNBC 307; gBRCA 220)

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2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-16

Title: Evaluating targets in localized and advanced breast cancer by sequencing tumor DNA at diagnosis and after progression (ELSA)

Gaba AG G, Powell SF F, Jennifer WL L, Megan LL L, Chun-Hung C, Lora BJ J and Ford JM M. Sanford Health, Fargo, ND; Sanford Health, Sioux Falls, SD and Stanford University School of Medicine, Stanford, CA.

Body: Background: Breast cancer therapy exerts strong selection pressure that shapes the evolution of the cancer. Despite the importance of these treatment-induced changes for the success of subsequent therapy, tumors have been rarely resampled and reanalyzed, with the exception of hematopoietic malignancies. The availability of next generation sequencing (NGS) has made it possible to get highly accurate sequencing that allows detection of mutations and other genetic alterations not only from tumor biopsies but also from circulating DNA fragments.

Objectives: To demonstrate the evolution of the molecular genotype of breast cancer as patients are diagnosed, treated, and upon relapse. This will be accomplished by NGS of the tumor at key time points during the natural history of the disease. The molecular profile at diagnosis will be compared to the profile at recurrence/metastasis and after treatment for metastasis.

Correlation with treatment response and adverse clinical outcomes will be determined.

Study Design and Eligibility: We have initiated a prospective observational study in patients with a new diagnosis of Stage I, II and III breast cancer and an ECOG performance status of 0, 1 or 2. Patients should be medically suitable to give informed consent for a biopsy or surgical procedure. Enrollment will occur at community-based cancer centers with inclusion of under-served populations.

Methodology: Tumor samples and blood samples are collected at - initial diagnosis/definitive surgery, first local relapse, diagnosis of metastasis and at first progression after treatment for metastatic disease. Molecular genotype will be analyzed from the tumor samples and from the circulating tumor DNA (ctDNA) in blood samples at each of the time points. ctDNA will also be assessed on blood samples collected annually till the diagnosis of metastasis and then more frequently at 3-6 month intervals in patients with high risk breast cancer.

Statistical Design/Size of study sample: Formal sample size calculations are not required for this study as by design, it is based on participation. There is no discrete endpoint that can be powered by sample size calculation for this study. We are screening all patients who are diagnosed with breast cancer at our facility. The proposed enrollment is 300 patients per year. The study launched in December 2015 at 4 of our community-based cancer centers. Current enrollments is 46 patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-17

Title: A Phase II randomized trial of pembrolizumab with carboplatin and gemcitabine for treatment of patients with metastatic triple-negative breast cancer (mTNBC)

Obeid E, Miller KD D, Sparano JA A, Blackwell K and Goldstein LJ J. Fox Chase Cancer Center, Philadelphia, PA; Indiana University, Indianapolis, IN; Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY and Duke University, Durham, NC.

Body: Background: Treatment for mTNBC is limited, and significant challenges persist in treating this disease, as outcomes remain largely dependent on chemotherapy without any effective targeted treatment. Pembrolizumab (MK-3475) is a highly selective, humanized monoclonal antibody against PD-1, blocking the negative immune regulatory signaling of the PD-1 receptor that is usually expressed by T-cells. Recent data showed that some patients with mTNBC may benefit from immune-based therapies (PD-1 or PD-L1 antibodies). Cumulative evidence suggest that stromal tumor infiltrating lymphocytes (sTILs) have a prognostic and predictive role in response to treatment in subsets of TNBC, particularly in response to carboplatin use. Preclinical data revealed that blocking PD-1/PD-L1 pathway in combination with platinum containing cytotoxic therapy improved response rates and survival. High levels of sTILs and an increased PD-L1 expression make mTNBC a candidate for PD-1–targeted therapy. As studies showed that the subset of TNBC with better response rates to carboplatin are heavily infiltrated with sTILs, pembrolizumab, becomes a very attractive drug to be tested in combination with carboplatin, with the goal of improving outcomes in mTNBC. A Phase II multicenter, randomized, trial has been initiated to evaluate the efficacy and safety of combining pembrolizumab with carboplatin and gemcitabine in patients with mTNBC.

Methods: A safety run-in will assess the safety and tolerability of combining pembrolizumab with carboplatin and gemcitabine in patients with mTNBC. Following the completion of the safety run-in, patients will be randomized 2:1 to receive pembrolizumab (200 mg IV) on day 1 along with carboplatin (AUC 2, day 1 and day 8, IV) plus gemcitabine (800 mg/m², day 1 and day 8, IV) of a 21-day cycle, or carboplatin plus gemcitabine (same aforementioned dose) alone. Patients will have histologically documented unresectable mTNBC. Prior systemic therapy for mTNBC, for up to 2 lines is allowed, and patients will have ECOG PS 0–2 and measurable disease (RECIST v1.1). Prior carboplatin/gemcitabine or cisplatin therapy is allowed in the adjuvant or neoadjuvant setting, as long as it occurred more than 12 months from the beginning of their enrollment. Subjects whose tumors progressed while on treatment with carboplatin or cisplatin are excluded. Known CNS disease (except asymptomatic treated metastases), autoimmune disease or prior immune checkpoint blockade therapy is an exclusion to enrollment on this trial. Primary endpoint is assessing the objective response rate according to RECIST v1.1 . Other endpoints include clinical benefit rate (CBR), progression-free survival (PFS), overall survival (OS), duration of response (DOR), and safety. Tumor biopsies will be obtained at baseline and just prior to initiation of cycle 3 to assess biomarkers of response and immune escape. PD-L1 expression will be evaluated in exploratory analysis with a planned assessment of response based on PD-L1 status. This trial will enroll 6-12 patients in the safety run-in portion, and 75 patients in the randomized part, at 7 sites in the United States. Clinical trial information: NCT02755272 www.clinicaltrials.gov.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-18

Title: RIBECCA - A phase IIIb, multi-center, open label study for women with estrogen receptor positive locally advanced or metastatic breast cancer treated with ribociclib (LEE011) in combination with letrozole

Fasching PA A, Decker T, Fehm T, Janni W, Kuemmel S, Lueftner D, Schneeweiss A, Schuler M, Guderian G, Schmidt-Wetzel S, Reiners R, Schubert J and Brucker S. University Hospital Erlangen, Erlangen, Germany; Oncology Ravensburg, Ravensburg, Germany; University Hospital Duesseldorf, Duesseldorf, Germany; University Hospital Ulm, Ulm, Germany; Breast Unit, Kliniken-Essen-Mitte, Essen, Germany; Med. Clinic for Haematology, Oncology, and Tumor Immunology, Charité Campus Benjamin Franklin, Berlin, Germany; National Center for Tumor Diseases, Heidelberg, Germany; University Hospital Essen, Essen, Germany; Novartis Pharma GmbH, Nuremberg, Germany and University Hospital Tuebingen, Tuebingen, Germany.

Body: Introduction:

The double blind, placebo-controlled MONALEESA-2 trial met its primary endpoint of clinically meaningful improvement in PFS at the pre-planned interim analysis for the combinatorial treatment of the cyclin-dependent kinase (CDK) 4/6-Inhibitor ribociclib and letrozole vs. letrozole monotherapy for postmenopausal, estrogen receptor positive (ER+), human epidermal growth factor receptor 2-negative (HER2-) women who were naïve to treatment for advanced disease.

Here we present the national, multi-center, open-label, single-arm phase IIIb trial RIBECCA assessing the efficacy and safety of the combination of ribociclib and letrozole in an additional population, accompanied by a set of exploratory objectives.

Methods:

Main inclusion criteria allow enrollment of women with metastatic or locally advanced breast cancer not amenable to curative treatment by surgery or radiotherapy, and histological or cytological confirmation of ER+, HER2- breast cancer, irrespective of their menopausal status.

The primary objective is to assess the Clinical Benefit Rate (CBR) after 6 months, the secondary objectives include progression free survival (PFS), overall survival (OS), safety and change in quality of life.

The exploratory objectives include the assessment of biomarkers (e.g. ctDNA, CTCs, miRNA) predictive for response and the occurrence of adverse events of special interest. The influence of CDK4/6-inhibition on the immune system will be another exploratory endpoint of this study.

Conclusion:

With a planned recruitment of 500 patients in about 100 trial sites the RIBECCA trial will contribute to a better understanding of the safety, efficacy and possibly mechanisms related to CDK4/6-inhibition through ribociclib.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-19

Title: A randomized phase II trial of fulvestrant with or without ribociclib after progression on aromatase inhibition plus cyclin-dependent kinase 4/6 inhibition in patients with unresectable or metastatic hormone receptor positive, HER2 negative breast cancer

Mundi PS S, Codruta C, Accordini MK K, Sparano J, Andreopoulou E, Vadhat LT T, Tiersten A, Esteva F, O'Regan R, Jain S, Mayer I, Forero A, Crew KD D, Hershman DL L and Kalinsky KM M. Columbia University Medical Center, New York, NY; Albert Einstein College of Medicine, New York, NY; Weill Cornell Medical Center, New York, NY; Mount Sinai School of Medicine, New York, NY; NYU Medical Center, New York, NY; University of Wisconsin School of Medicine, Madison, WI; Northwestern, Chicago, IL; Vanderbilt-Ingram Cancer Center, Nashville, TN and University of Alabama-Birmingham, Birmingham, AL.

Body: Background

Cyclin dependent kinase 4 and 6 inhibitors (CDK4/6i), including palbociclib and ribociclib (R), have demonstrated remarkable benefit in progression free survival (PFS) in patients (pts) with hormone receptor positive (HR+), HER2- metastatic breast cancer (MBC) when combined with anti-estrogen therapy. Switching between anti-estrogen therapies at disease progression is standard of care in the treatment of HR+ MBC. We evaluate the strategy of switching anti-estrogen therapy to fulvestrant (F) and maintaining CDK4/6 inhibition with R in pts with HR+, HER2- MBC who have progressed on an aromatase inhibitor (AI) + CDK4/6i.

Trial Design

Phase II, multi-center, randomized, double-blind, placebo-controlled trial to evaluate F +/- R in pts with HR+, HER2- MBC who have previously progressed on any AI + CDK4/6i. Pts can be screened and registered at two different time points:

Scenario 1: Before receiving any CDK4/6i

Scenario 2: At the time of progression of disease (POD) while being treated with an AI + CDK4/6i

In scenario 1, the study will provide pts with letrozole + R, but pts will not be randomized until they demonstrate POD. At randomization, pts will be assigned 1:1 to either a) F + R or b) F + placebo, with treatment given in 4-week cycles. F will be given as a 500 mg dose intramuscularly every 2 weeks for 3 times and then every 4 weeks, as per standard of care. R or placebo will be given orally at 600 mg daily, 3 weeks on/1 week off. CT scans and bone scan are to be performed prior to every third cycle. Serum and whole blood samples and optional tissue biopsies for biomarker assessment will be performed prior to study treatment (scenario 1), prior to randomization to R +/- F, and when the patient goes off study.

Main Eligibility Criteria:

1. Age \geq 18 years with unresectable or metastatic BC
2. Estrogen and/or progesterone receptor positive, HER2 negative, as per ASCO-CAP
3. Postmenopausal status or receiving ovarian suppression
4. Measurable or unmeasurable disease; stable CNS disease allowed
5. No clinically significant cardiac disease
6. No concomitant CYP3A4/5 inducer or inhibitor

Specific Aims

Primary: Progression free survival (PFS), defined as the time from randomization to POD or death.

Secondary: Objective response rate (ORR), clinical benefit rate (CBR = ORR + stable disease rate), overall survival (OS), and duration of response. Pts in scenario 1 will also be assessed for PFS, OS, CBR, and safety while receiving AI + R (pre-randomization).

Biomarker assessment will include amplification of cyclin D1 and cyclin E, phosphoRb and TK1 expression, Rb1 and p16 loss, and ctDNA for ESR1 and PIK3CA mutations.

Target Accrual

132 pts accrued from 11 academic medical centers in the U.S, with a goal of completing accrual in two years (~60 to 72 pts in each scenario).

Statistical Methods

Assuming a median PFS of 3.8 months with F alone, we predict that F + R will lead to a median PFS of at least 6.5 months. A one-sided log-rank test with a sample size of N=120 and alpha=0.025, achieves 80% power to detect a difference in PFS of 2.7

months. N=132 pts allows for a 10% drop-out rate.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-20

Title: Phase IIB study of neoadjuvant panitumumab combined with carboplatin and paclitaxel (PaCT) for anthracycline-resistant triple-negative breast cancer (TNBC)

Lim B, Helgason T, Hess KR R, Piwnica-Worms H, Yang W, Adrada BE E, Rauch GM M, Gilcrease M, Symmans FW W, Huo L, Mittendorf EA A, Thompson A, Stacy M-TL L, Debu T and Ueno NT T. The University of Texas, MD Anderson Cancer Center, Houston, TX.

Body: BACKGROUND: Approximately 50% of patients with TNBC treated with standard taxane/anthracycline-based neoadjuvant chemotherapy (NACT) have chemo-insensitive disease (CID), i.e., residual disease burden (RCB)-II/III at the time of surgery, and 40-80% of patients develop recurrence within 3 years. Recent developments in molecular profiling have identified subsets of TNBC with distinct, targetable molecular features. We developed a clinical trial to identify and characterize CID (ARTEMIS: A Randomized, TNBC-Enrolling trial to confirm Molecular profiling Improves Survival). In ARTEMIS, patients with localized TNBC will undergo a pretreatment biopsy, then begin anthracycline-based NACT. During NACT, we use molecular profiling and response assessment to identify CID and allocate patients to alternative therapies to overcome CID. Epidermal growth factor receptor (EGFR) is overexpressed in 25-30% of TNBC. In preclinical studies, suppression of EGFR signaling has shown efficacy in controlling cancers through suppression of the stem cell population, enhanced apoptosis via MAPK/PI3K signaling, and modulation of epithelial-mesenchymal transition (EMT). Moreover, in a phase II trial of triple negative inflammatory breast cancer, neoadjuvant PaCT yielded significantly higher pathologic complete response (pCR) rates than historic control. Taken together, we hypothesize that using PaCT to suppress EGFR in TNBC will enhance the pCR rate.

OBJECTIVES: Primary objective: determine pCR and RCB-0/I rates in TNBC patients with CID given PaCT. Secondary objective: determine the benefit of using baseline genomic signatures to develop an alternative second phase of NACT.

TRIAL DESIGN AND STATISTICAL METHODS: Patients with >10% volume reduction for non-CID or <80% for CID will enroll in a biomarker-guided, experimental, nonrandomized phase II study and be given PaCT (panitumumab 2.5 mg/kg, carboplatin AUC 5, paclitaxel 80 mg/m²). Because pCR rates in pts with CID with additional cycles of taxane-based therapy are low (~5%), a 20% response rate (RCB-0 or RCB-I) will be considered clinically meaningful. A two-stage Gehan-type design will be employed. If at least 1 of 14 patients responds, 23 more patients will be added, for a total of 37 patients. This design has a 49% chance of terminating after the first stage if the true response rate is 0.05, 23% if the rate is 0.10, 10% if the rate is 0.15, and 4% if the rate is 0.20. If accrual continues to the second stage and 37 patients are enrolled, the 95% confidence interval for a 0.20 response rate will be 0.10 to 0.35.

BRIEF ELIGIBILITY CRITERIA: *Inclusion:* localized TNBC; enrolled in ARTEMIS trial; adequate organ, bone marrow, and cardiac parameters; *Exclusion:* pregnant or lactating, known or suspected metastasis.

CORRELATIVE SCIENCE: Circulating tumor cells (CTCs) and cell free (cf) DNA in baseline and subsequent blood samples, EGFR expression (immunohistochemistry), stem cell/EMT/apoptosis marker changes in tissue and CTCs, PD-L1 glycosylation for EGFR sensitivity.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-21

Title: Phase 2 study of investigational TORC1/2 inhibitor TAK-228 with fulvestrant in women with ER-positive/HER2-negative advanced or metastatic breast cancer that has progressed during or after aromatase inhibitor therapy

García-Sáenz JA A, Carrasco E, Kneissl ML L, Zohren F and Martin M. Hospital Clínico Universitario San Carlos, Madrid, Spain; GEICAM (Spanish Breast Cancer Group), San Sebastian de los Reyes, Madrid, Spain; Millennium Pharmaceuticals, Inc., a Wholly Owned Subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, MA and Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain.

Body: Standard therapy for ER-positive tumors in the adjuvant and metastatic settings is antiestrogen therapy, including aromatase inhibitors (AI); however, resistance is common. These tumors may respond to alternative second-line anti-estrogen therapies such as fulvestrant but response durations are often short. Preclinical and clinical studies suggest that simultaneous inhibition of ER and PI3K/mTOR could prevent/delay the emergence of hormone-independent cancer cells thereby improving patient (pt) outcomes. This study will test whether fulvestrant plus TAK-228, a dual TORC1/2 inhibitor, can overcome endocrine therapy resistance in ER-positive breast cancer. This is an open-label, randomized, 3-arm, phase 2 study of continuous once-daily TAK-228 (oral, 4 mg) or once-weekly TAK-228 (oral, 30 mg) plus fulvestrant (500 mg intramuscularly on d1 and d15 of cycle 1 [loading regimen] then d1 of each subsequent 28-d cycle), compared with fulvestrant alone, in pts with advanced or metastatic ER-positive/HER2-negative breast cancer that has progressed during/after AI therapy. Pts will be randomized 1:1:1 to the 3 arms and stratified at randomization via presence or absence of visceral metastasis, prior hormonal therapy sensitivity, and prior exposure to CDK 4/6 inhibitors. Pts will receive study drug(s) until progressive disease (PD), unacceptable toxicity, or consent withdrawal. Postmenopausal women ≥ 18 yrs old with local histological confirmation of ER-positive/HER2-negative metastatic or advanced breast cancer; with measurable disease; ECOG status 0–1; PD during/after prior AI therapy (defined as progression ≤ 12 mos after discontinuing adjuvant therapy or ≤ 1 mo after discontinuation in the metastatic setting) and adequate organ function are eligible. Exclusion criteria include prior therapy with mTOR inhibitors, PI3K inhibitors, dual PI3K-mTOR inhibitors, AKT inhibitors, or fulvestrant; prior treatment with >1 line of chemotherapy for metastatic breast cancer; experienced recurrent or progressive disease on >2 endocrine therapies for metastatic breast cancer; or significant previous/existing cardiac conditions. This study aims to determine the efficacy (primary endpoint: PFS; secondary endpoints: OS, TTP, ORR), safety and tolerability of daily and weekly TAK-228 plus fulvestrant compared with fulvestrant alone. The distribution of PFS will be analyzed via the Kaplan-Meier method. The primary hypothesis (TAK-228 plus fulvestrant can improve median PFS to 8 mos [hazard ratio, HR 0.5] vs fulvestrant-alone median PFS of 4 mos) is to be tested at the 0.10 significance level (2-sided; dropout rate 10%). A total of 72 PFS events are needed for each pair-wise comparison; p-values from a stratified log-rank test and HRs will be presented. The safety profile and clinical laboratory parameters (and/or change from baseline) for all scheduled measurements over time will be summarized by descriptive statistics. Approximately 153 pts (51 pts per arm) will be enrolled from approximately 55 study centers in North America and Spain; to date, no pts have been enrolled. For more information, please contact Michelle Kneissl at michelle.kneissl@takeda.com.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-22

Title: NCT02456857: A phase II trial of liposomal doxorubicin, bevacizumab and everolimus (DAE) in patients (pts) with localized triple-negative breast cancer (TNBC) with tumors predicted insensitive to standard neoadjuvant chemotherapy (NACT)

Moulder S, Hess K, Rauch M, Astrada B, Litton J, Mittendorf E, Ueno N, Tripathy D, Lim B, Piwnica-Worms H, Thompson A and Symmans WF. University of Texas, MD Anderson Cancer Center.

Body: **BACKGROUND:** Approximately 50% of TNBC pts treated with standard taxane/anthracycline-based NACT will have chemo-insensitive disease (CID) manifested as extensive residual disease (RCB-II or III) at the time of surgery. 40-80% of these pts will develop recurrence within 3 years of initial diagnosis. Recent advances in molecular profiling have identified subsets of TNBC with distinct, targetable molecular features. We developed a clinical trial to identify and characterize CID (**ARTEMIS: A Randomized, TNBC Enrolling trial to confirm Molecular profiling Improves Survival**). In the ARTEMIS trial, treatment naïve pts with localized TNBC undergo a pretreatment biopsy and then immediately start their initial phase of anthracycline-based chemotherapy so that the results of the molecular characterization are used in combination with response assessment (clinical exam/diagnostic imaging) to identify CID and inform the second phase of NACT, thus using a 'second hit' strategy in the middle of NACT to overcome drug resistance. The mesenchymal subtypes of TNBC have a high incidence of PI3K pathway activation. Preclinical models demonstrated response to PI3K inhibitors in this subtype. Metaplastic breast cancers make up ~30% of tumors characterized as 'claudin-low/mesenchymal' by gene signature and are also associated with a high rate of PI3K activating molecular aberrations. A combination regimen of liposomal doxorubicin, bevacizumab and the mTOR inhibitors temsirolimus or everolimus (DAT or DAE) demonstrated response (including durable complete responses) in metastatic metaplastic breast cancer.

PRIMARY OBJECTIVE: Determine the rate of pathologic complete response (pCR/RCB-0) or minimal residual disease (RCB-I) after 4 cycles of DAE for treatment of mesenchymal TNBC deemed to be CID through the ARTEMIS trial

TRIAL DESIGN AND STATISTICAL METHODS: Only pts deemed to have mesenchymal CID on the ARTEMIS trial can enter this non-randomized phase II study. Realizing that pts without response to their initial cycles of chemotherapy have very low chance (5%) of achieving pCR with additional cycles of chemotherapy, it would be clinically meaningful to see pCR in this pt population improved to 20%. Counting pCR (RCB-0) or RCB-I as response, a two-stage Gehan-type design will be employed with 14 pts in the first stage. If at least one pt responds, 23 more pts will be added for a total of 37 pts. This design has a 49% chance of terminating after the first stage if the true response rate is 0.05, 23% chance if the true rate is 0.10, 10% if the true rate is 0.15 and 4% if the true rate is 0.20. If accrual continues to the second stage and a total of 37 pts are enrolled, the 95% confidence interval for a 0.20 response rate will extend from 0.10 to 0.35.

BRIEF ELIGIBILITY CRITERIA: *Inclusion:* localized TNBC enrolled onto ARTEMIS trial, adequate organ, bone marrow and cardiac parameters *Exclusion:* metastatic disease, pregnant or lactating pts, medical illness that increases chance of moderate to severe toxicity

CORRELATIVE SCIENCE: Correlate vimentin expression by IHC, mesenchymal signatures and PI3K pathway aberrations with response.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-02-01

Title: Photodynamic therapy for the treatment of primary breast cancer: Preliminary results of a phase I/IIa clinical trial

Banerjee SM M, Malhotra A, El-Sheikh S, Tsukagoshi D, Tran-Dang M-a, Mosse A, Parker S, Davidson TI I, Williams NR R, Bown S and Keshtgar MR R. Royal Free London NHS Foundation Trust, London, United Kingdom; University College London, Div. Surgery and Interventional Science, London, United Kingdom and University College London, Surgical & Interventional Trials Unit, Div. Surgery and Interventional Science, London, United Kingdom.

Body: Background: Photodynamic therapy (PDT) has been used to treat skin metastases from breast cancer. We investigated the use of PDT for the treatment of primary breast cancer.

Trial design: Phase I/IIA, open label, non-randomised, single site, light dose escalation trial in patients with primary breast cancer using verteporfin as the photosensitiser. Verteporfin 0.4mg/kg bodyweight is injected intravenously 60-90 minutes before laser activation through a thin optical fibre inserted percutaneously through a needle positioned under ultrasound guidance under local anaesthesia. The light dose is escalated from 20J to a maximum of 50J in intervals of 10J.

Eligibility criteria: Patients aged 30 or over and have opted for mastectomy or wide local excision as primary treatment are included. The tumour should be uni-focal invasive ductal breast carcinoma, or discrete uni-focal site, within a multifocal invasive ductal carcinoma, in a single breast. Participants should not have confirmed distant metastases. The exclusion criteria include: patients who are not undergoing surgery as their primary treatment; patients undergoing surgery for DCIS without invasive breast cancer; lobular cancers and necrotic tumours; patients who have porphyria or are sensitive to verteporfin; patients who have severe cardiovascular disease or severe uncontrolled systemic disease (e.g. hepatic impairment); male breast cancer patients; pregnant or lactating patients; patients taking primary endocrine therapy or taking an experimental medicine as a part of a trial.

Aims: We aim to establish the minimum light dose required to induce an area of necrosis with a diameter of at least 12mm perpendicular to the optical fibre; or to achieve a plateau with no increase in diameter of necrosis with increasing light dose. Secondary objectives are to examine the effect of Photodynamic Therapy on the abnormal breast tissue and study if necrosis extends to normal breast tissue. We will be assessing the role of MRI in predicting the response to treatment by measuring the diameter of tumour and the zone of necrosis before and after PDT and confirming it with histological findings. We will be monitoring the number of adverse events arising from the PDT.

Statistical Methods: The sample size of the study is based on a standard 3+3 dose-escalation algorithm. Statistical analysis will be descriptive in nature with no formal statistical inference. Summary statistics and analysis will be provided for all patients who receive the study treatment, by dose level for each dosing regimen.

Accrual Results: 11 patients have been enrolled and completed the trial up to June 2016 with primary end point achieved and no adverse side-effects to treatment seen.

Target Accrual: 21 patients.

Acknowledgement: Royal Free Charity.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-02-02

Title: A randomized, open-label, multicenter, phase 1b/2 study of eribulin mesylate in combination with PEGylated recombinant human hyaluronidase in patients with human epidermal growth factor receptor 2-negative, high-hyaluronan metastatic breast cancer

Alvarez RH H, Savulsky C, Almonte A, Xing D, Lokker N and Chondros D. Cancer Treatment Centers of America, Southeastern Regional Medical Center, Atlanta, GA; Eisai Ltd., Hatfield, England, United Kingdom; Eisai Inc., Woodcliff Lake, NJ and Halozyme Inc., San Diego, CA.

Body: Eribulin (ERI), a non-taxane microtubule inhibitor, is approved for treatment in patients (pts) with advanced or metastatic breast cancer (MBC) who have received ≥ 1 (European Union) or ≥ 2 (United States) prior chemotherapy regimens for metastatic disease, including an anthracycline and taxane in either the adjuvant or metastatic setting. In a phase 2 study, ERI has demonstrated single-agent activity in first line treatment of pts with human epidermal growth factor receptor 2-negative (HER2-) MBC. Hyaluronan (HA) is a major component of the tumor microenvironment, specifically within the stroma, and high levels of HA are associated with disease progression. In HA-High, triple-negative breast cancer (TNBC) xenografts, the anti-tumor effect of ERI was enhanced by addition of PEGylated recombinant human hyaluronidase (PEGPH20). PEGPH20 is an engineered enzyme under investigation for use in combination with anticancer therapies in pts with tumors that accumulate HA. PEGPH20 acts by temporarily degrading HA to reduce tumor interstitial pressure and decompress blood vessels, thus increasing access of anticancer agents and immune cells to tumor sites. This randomized, open-label, multicenter, study of ERI in combination with PEGPH20 vs ERI alone was designed to evaluate safety and tolerability and efficacy in pts with HER2-, HA-High, MBC previously treated with 0-1 systemic (cytotoxic or targeted) anticancer therapy.

The trial was activated in June 2016 and will enroll approximately 96 pts (aged ≥ 18 yrs; n=6-18 in phase 1b; n=84 randomized to obtain 80 evaluable pts in phase 2). Pts with measurable disease of ≥ 1 lesion ≥ 10 mm in long-axis diameter (nonlymph nodes) or ≥ 15 mm in short-axis diameter (lymph nodes) with ECOG PS of 0 or 1 will be included. Pts will be excluded for having had adjuvant chemotherapy within the past 6 months, hormonal/biological therapy within the past 3 wks, or radiation or small molecule targeted therapy within the past 2 wks. Pts will also be excluded if they were previously treated with ERI or any hyaluronidase agent.

Phase 1b includes at least 1 initial safety run-in cohort in which 6 pts (any HA level) will receive PEGPH20 3 $\mu\text{g}/\text{kg}$ intravenous (IV) on days -1 and 7 of a 21-day cycle, each dose followed approximately 24 (± 4) hours later with ERI 1.4 mg/m^2 IV on days 1 and 8. Dose-limiting toxicity (DLT) will be assessed in the first cycle. The primary endpoint in phase 1b is the recommended phase 2 dose (RP2D) of the ERI and PEGPH20 combination, defined as the maximum dose at which ≤ 1 pt experiences a DLT. Alternatively, modified dose levels will be evaluated in additional cohorts.

In phase 2, HA-High pts will be stratified by TNBC vs other HER2- status and randomized to receive RP2D combination treatment or ERI alone. Pts with clinical benefit will remain on ERI or both study drugs until unacceptable toxicity or disease progression. The primary endpoint in phase 2 is objective response rate. Secondary endpoints include progression-free survival and overall survival. Exploratory endpoints include clinical benefit rate, disease control rate, and duration of response.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-02-03

Title: Pilot study of zirconium-89 bevacizumab positron emission tomography for imaging angiogenesis in patients with inflammatory breast carcinoma receiving preoperative chemotherapy

Jacene HA A, Overmoyer B, Schlosnagle EJ J, Abbott A, Yeh E, Paolino J, Goel S, Culhane A, Bellon JR R, Nakhlis F, Hirshfield-Bartek J and Van den Abbeele A. Dana-Farber Cancer Institute; Brigham and Women's Hospital and Co-First Authors Contributing Equally to This Work.

Body: Background: Inflammatory breast cancer (IBC) continues to have a poor prognosis despite standard tri-modality treatment with chemotherapy, mastectomy and radiation. Current methods of assessing primary tumor response (i.e., clinical exam and breast magnetic resonance imaging [MRI]) are limited for distinguishing residual tumor from responsive disease because of persistent morphologic changes in the breast. Therefore, the inability to accurately assess tumor response during treatment often results in the continuation of ineffective systemic chemotherapy until definitive pathologic evaluation at mastectomy. IBC has a highly angiogenic phenotype which is believed to play a role in this tumor's aggressiveness. The novel radiotracer Zirconium-89 (⁸⁹Zr)-bevacizumab was developed for imaging tumor angiogenesis with PET. We hypothesize that, as an imaging biomarker of angiogenesis, ⁸⁹Zr-bevacizumab-PET/CT is a more specific noninvasive functional imaging modality for detecting the presence of tumor angiogenesis compared to current diagnostic methods and will serve as a predictor of response to therapy in patients (pts) with IBC.

Methods: Pts with newly diagnosed HER2neg IBC who will receive preoperative chemotherapy are eligible for this pilot study. ⁸⁹Zr-bevacizumab-PET/CT, breast MRI and FDG-PET/CT are performed before, after 2 cycles, and at the completion of preoperative therapy. Biopsies of primary IBC tumors are obtained prior to and after 2 cycles of preoperative therapy. At the completion of preoperative therapy, pts proceed to mastectomy or biopsy if ineligible to proceed to mastectomy based on current standards for assessing primary tumor response, i.e., clinical exam, breast MRI and lack of systemic progression. At the time of mastectomy, standard evaluation of the surgical specimen will determine pathologic response of IBC to preoperative chemotherapy. A research sample will be collected if residual cancer is present at the time of mastectomy for histologic evaluation of tumor angiogenesis.

Objectives/Correlatives: The primary objective is to determine feasibility of ⁸⁹Zr-bevacizumab-PET/CT imaging in pts with IBC. The primary endpoint is assessment of radiolabeling of chelated bevacizumab and number of successfully acquired ⁸⁹Zr-bevacizumab-PET/CT scans. Correlative studies will be performed on IBC tissue to assess extent of angiogenesis including microvessel density, vessel diameter, vascular pericyte coverage and tumor VEGF levels. Secondary objectives are: 1) To determine if ⁸⁹Zr-bevacizumab accumulation in primary IBC tumors correlates with the extent of angiogenesis determined by correlative analysis on IBC tissue; 2) To assess the predictive value of ⁸⁹Zr-bevacizumab-PET/CT after 2 cycles and at the end of preoperative therapy for determining pathologic response at mastectomy as given by residual cancer burden.

Statistics: This is an accrual, not statistical based, feasibility justification. Planned sample size is 10 in order to make a preliminary statement about feasibility and ability for ⁸⁹Zr-bevacizumab-PET/CT to serve as a surrogate in vivo biomarker of tumor angiogenesis and response to preoperative chemotherapy.

Clinical Trial Information: NCT01894451.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-03-01

Title: NRG oncology/NSABP B-51/RTOG 1304: A phase III superiority clinical trial designed to determine if chest wall and regional nodal radiotherapy (CWRNRT) post mastectomy (Mx) or the addition of RNRT to breast RT post breast-conserving surgery (BCS) will reduce invasive cancer events in patients (pts) with positive axillary (Ax) nodes and convert to ypN0 after neoadjuvant chemotherapy (NC)

Mamounas EP P, Bandos H, White JR R, Julian TB B, Khan AJ J, Shaitelman SF F, Torres MA A, Vicini FA A, Ganz PA A, McCloskey SA A, Paik S, Gupta N, Li XA Allen, DiCostanzo DJ J, Curran, Jr WJ J and Wolmark N. NRG Oncology/NSABP (NSABP Legacy Trials Are Now Part of the NRG Oncology Portfolio), Pittsburgh, PA; UF Health Cancer Center at Orlando Health, Orlando, FL; University of Pittsburgh, Pittsburgh, PA; NRG Oncology/RTOG, Philadelphia, PA; Ohio State University, Columbus, OH; Allegheny Health Network Cancer Institute, Pittsburgh, PA; Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; University of Texas MD Anderson Cancer Center, Houston, TX; Winship Cancer Institute Emory University, Atlanta, GA; St. Joseph Mercy Oakland, Pontiac, MI; University of California at Los Angeles, Los Angeles, CA; Severance Biomedical Sci Inst and Yonsei Univ College of Medicine, Seoul, Korea; Medical College of Wisconsin, Milwaukee, WI and The Ohio State University Wexner Medical Center, Columbus, OH.

Body: Background:

This phase III post-NC trial evaluates if CWRNRT post Mx or whole breast irradiation (WBI) with RNRT after BCS significantly reduces the invasive breast cancer recurrence-free interval (IBC-RFI) rate in pts presenting with positive Ax nodes that are pathologically negative after NC. Secondary aims are OS, LRRFI, DRFI, DFS-DCIS, and second primary cancer, as well as comparing RT effect on cosmesis in reconstructed Mx pts.

Correlative science studies examine RT effect by tumor subtype, molecular outcome predictors for residual disease pts, and predictors for the degree of reduction in loco-regional recurrence.

Methods:

Clinical T1-3, N1 IBC pts with positive Ax nodes (FNA or core needle biopsy) complete ≥ 8 wks of NC (anthracycline and/or taxane). HER2-positive pts receive anti-HER2 therapy (tx). After NC, BCS or Mx is performed with a sentinel node biopsy (≥ 2 nodes) and/or Ax dissection with histologically negative nodes. ER/PR and HER2 neu status before NC is required. Pts receive required systemic tx. Radiation credentialing with a facility questionnaire and a case benchmark is required. Randomization for Mx pts is to no CWRNRT or CWRNRT and for BCS pts to WBI or WBI+RNRT.

Statistics:

1636 pts to be enrolled over 5 yrs with definitive analysis at 7.5 yrs. Study is powered at 80% to test that RT reduces the annual hazard rate of events for IBCR-FI by 35% for an absolute risk reduction in the 5-yr cumulative rate of 4.6%. Intent-to-treat analysis with 3 interim analyses at 43, 86, and 129 events, with a 4th/final analysis at 172 events will occur. Accrual as of 6/13/16 is 356. Pt-reported outcomes focusing on RT effect will be obtained from 736 pts before randomization and at 3, 6, 12, and 24 months.

Contacts:

Protocol: CTSU member website <https://www.ctsu.org>. Questions: NRG Oncology Pgh Clin Coord Dpt: 1-800-477-7227 or ccd@nsabp.org. Pt entry: OPEN at <https://open.ctsu.org> or the OPEN tab on CTSU member website.

Support: U10 CA-2166; -180868, -180822; -189867; Elekta.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-03-02

Title: Feasibility of assessing radiation response with MRI/CT directed preoperative accelerated partial breast irradiation in the prone position for hormone sensitive early stage breast cancer

White J, McElroy S, Seykon A, Wei L, Bazan J, Yang X, DiCostanzo D, Gupta N and Knopp M. The Ohio State University Comprehensive Cancer Center, Columbus, OH; Center for Biostatistics, The Ohio State University Comprehensive Cancer Center, Columbus, OH and Wright Center for Innovation, The Ohio State University, Columbus, OH.

Body: Background: Accelerated partial breast irradiation (APBI) delivers adjuvant radiation (RT) to the 1-2 cm of the breast at highest risk for recurrence surrounding the lumpectomy (L) cavity over 5-8 days and is an alternative to standard whole breast irradiation for hormone sensitive (HS) stage 1 (T1, N0) breast cancer (BC) based on 2 randomized controlled trials. External beam methods for APBI are common but have notable inherent drawbacks that include: inter-fraction inaccuracy due to patient setup based on anatomy, intra fraction error related to patient or respiratory motion, and inaccurate geometric targeting by relying on L cavity position instead of the tumor position. Postoperative RT has other limitations including delivery in the setting of disrupted blood or lymphatic supply that may be suboptimal for radio sensitivity and it eliminates observation of radiation-induced tumor response. MRI is an established tool for measuring BC extent and response from neoadjuvant systemic therapy. It's hypothesized that MRI directed pre-operative APBI using intensity modulated radiotherapy (IMRT) with image guidance (IGRT) will improve RT delivery, and that MRI features can be identified to correlate with pathologic radiation response.

Trial Design: There are 2 cohorts to this single arm prospective trial. The first cohort is for establishing and verifying patient flow and image fusion between MRI, CT and RT planning. In the second cohort eligible patients will receive preoperative APBI 38.5 Gy in 10 fractions BID with IMRT, IGRT in the prone position using MRI defined targets fused to CT treatment planning.

Eligibility: For cohort 1 it is HS Stage 1 BC that has completed CT in prone position for RT planning. Eligibility for cohort 2 requires: age > 60 yo, clinical stage 1 BC, HS, HER2 negative, intending L, clinically negative axilla verified by ultrasound, able to tolerate the prone position, and MRI with contrast.

Specific aims: To determine the reproducibility of MRI directed preoperative APBI based on meeting 3 criteria: ability to define RT targets by MRI, quality of RT plans and completion of treatment (APBI and surgery). Additional aims include assessing toxicity, cosmetic outcome, local regional cancer control and collection of tissue for correlative studies.

Statistical methods: The optimal two-stage design by Simon is used. Sample size for cohort 2 is based on the first endpoint. 19 eligible patients will be required in the first stage; if 3 or more treatments are scored unacceptable, then early stopping will be recommended. Otherwise, accrual will continue to a total accrual of 30. If ≥ 4 of 30 treatments are scored unacceptable, the technique will be considered not reproducible, and a Phase II study will not be pursued. Under the null hypothesis of an 80% reproducibility rate, this two-stage design has an expected sample size of 24.4.

Patient accrual to cohort 1 is 2 and targeted accrual is 3. **Patient accrual** to Cohort 2 is 0 and targeted accrual is 30.

Contact information: Sohyun Mc Elroy (Sohyun.McElroy@osumc.edu) or Julia White (Julia.White@osumc.edu)

Funding source: Susan G Komen Breast Cancer Foundation Grant # GRT00035216.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-03-03

Title: A randomized trial of 15 fraction vs 25 fraction pencil beam scanning proton radiotherapy after mastectomy in patients requiring regional nodal irradiation

Mutter RW W, Park SS S, Hieken TJ J, Vargas CE E, Mei-Yin PC C, Kathryn RJ J, Hector VR R, Kimberly CS S, Elizabeth YS S and Daniel VW W. Mayo Clinic, Rochester, MN.

Body: Background: Post-mastectomy radiotherapy improves survival in women with node-positive breast cancer. Pencil beam scanning proton therapy is attractive due to potential to reduce the dose to the heart and lungs compared with traditional photon techniques while improving conformality and limiting skin dose compared with passively scanned proton therapy. The optimal dose and fractionation for pencil-beam scanning proton therapy remains unknown.

Trial Design: This is a multi-center open label phase II randomized controlled trial to determine the safety of 15 fraction vs 25 fraction pencil beam scanning proton radiotherapy after mastectomy in patients requiring regional nodal irradiation.

Eligibility Criteria: Patients \geq 18 years with primary, non-inflammatory invasive breast cancer who have undergone mastectomy with or without immediate reconstruction and chest wall and regional nodal irradiation planned.

Aims: To determine whether the 24 month complication rate (defined as grade 3 or greater late adverse events, and unplanned surgical intervention in patients who undergo mastectomy with reconstruction) of 15 fraction chest wall and regional node pencil beam scanning proton radiotherapy is acceptable relative to 25 fraction chest wall and regional nodal pencil beam scanning proton radiotherapy and worthy of further investigation.

Statistical methods: The study is designed as a non-inferiority/superiority "hybrid" design using the approach of Freidlin et al. It is Using a one-sided type I error rate of 0.05 (corresponding equivalently to constructing a 1-sided 95% confidence limit), 72 evaluable patients will have 80% power to reject the null hypothesis that the 24-month complication rate in the experimental arm is higher than that of the control arm by more than 10% (i.e. rule out inferiority) under the alternative hypothesis that the complication rate in the experimental arm is 5% less than that of the control arm (i.e. superiority). However, the design will have only 41% power when the two treatment arms are equivalent (i.e. the complication rate is 10% for both arms).

Accrual: The study opened in June 2016. Five of a planned eighty-two patients have been accrued to date.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-04-01

Title: Study on the usefulness of the skin wound therapeutic agent 'Neo dermal activator' in breast cancer surgery

Lim SM, Kim SI, Park SH, Park HS, Kim JY and Kim JH. Yonsei University Health System, Seoul, Korea.

Body: Background : Wound infection after breast cancer surgery is considered to be factors that increase the discomfort of the patient and increase of the extension and the use of antibiotics in the treatment time period, in particular breast cancer postoperative wound infection resulting slow the implementation of adjunctive therapy such as radiation therapy or chemotherapy after surgery can cause, it is important to effectively prevent, there has been a development of a variety of skin wound therapeutic agent to reduce them. The purpose of this study was to evaluate the effects of the wound dressing with existing wound dressing *Neo dermal activator* compared to the patients receiving preoperative chemotherapy.

Methods : A total of 54 breast cancer patients who recieved BCS & mastectomy devided two group(each 27 patients) through the random assignment between Jan, 2016 and June, 2016. Day one weeks after surgery to examine the wound infection rate (surgical site infection, SSI rate). After six months of outpatient surgery visits to check the wounds of patients, using the VAS records the scar of the wound satisfaction. For patients who underwent radiation therapy, check out the time it took to begin radiation therapy after surgery.

All tests were two-sided. All statistical analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, Illinois, USA).

Eligibility criteria is :

- one side breast cancer
- not inflammatory breast cancer
- recieved BCS and mastectomy only (without immediate reconstruction surgery)
- stage IV breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT2-04-02

Title: Comparison of axillary sentinel lymph node biopsy versus no axillary surgery in patients with early-stage invasive breast cancer and breast-conserving surgery: A randomized prospective surgical trial. The Intergroup-Sentinel-Mamma (INSEMA)-trial

Reimer T, von Minckwitz G, Loibl S, Hildebrandt G, Nekljudova V, Schneider-Schranz C and Gerber B. Breast Center University of Rostock; German Breast Group and Radiotherapy University of Rostock.

Body: Background:

Currently, axillary surgery for breast cancer is considered as staging procedure that does not seem to influence breast cancer mortality, since the risk of developing metastasis depends mainly on the biological behaviour of the primary. Based on this, the postsurgical therapy should be considered on the basis of biologic tumor characteristics rather than nodal involvement.

Trial design:

The goal of INSEMA is to show that early-stage breast cancer patients with reduced extent of axillary surgery are not inferior regarding invasive disease-free survival (IDFS) outcome. Patients with planned breast-conserving surgery (BCS) will be first randomized (1:4 ratio) to either no axillary surgery or axillary sentinel lymph node biopsy (SLNB). Patients with SLNB and pN+(sn) status will be secondly randomized (1:1 ratio) to either SLNB alone or completion axillary lymph node dissection (ALND) in cases with less than three involved nodes (one or two macrometastases).

Primary objective:

-IDFS after BCS (non-inferiority question)

Inclusion criteria:

-Written informed consent

-Histologically confirmed unilateral primary invasive carcinoma of the breast (core biopsy)

-Age at least 35 years

-Preoperative imaging techniques with estimated tumor size of maximal 5 cm (iT1/iT2 irrespective of hormone sensitivity or HER2 status)

-Clinically and sonographically tumor-free axilla prior to core biopsy

-In cases with cN0 and iN+, a negative core biopsy or fine needle aspiration biopsy of the suspected lymph node is required

-No clinical evidence for distant metastasis (M0)

-Planned breast-conserving surgery (R0 resection) with postoperative external whole-breast irradiation (conventional fractionation or hypofractionation)

Statistics:

The calculated total case number for per-protocol analyses is 6,740 (5,940 German and 800 Austrian patients), the expected total number of randomized patients is 7,095.

Time lines:

-First patient in: September 2015

-Last patient in: August 2019

-Final analysis: End of 2024

Present accrual: In June 2016, more than 1.000 patients were recruited in Germany and Austria.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-01-01

Title: Comparative performance of surveillance mammography and breast MRI in women with a history of breast cancer

Wernli KJ J, Ichikawa L, Kerlikowske K, Bush M, Johnson D, Buist DSM S M, Brandzel SD D, DeMartini WB B, Henderson L, Nekhlyudov L, Onega T, Sprague B and Miglioretti DL L. Group Health Cooperative, Seattle, WA; University of California, San Francisco, San Francisco, CA; University of Wisconsin School of Medicine, Madison, WI; Brigham and Women's Hospital, Boston, MA; Dartmouth Medical School, Hanover, NH; University of Vermont, Burlington, Burlington, VT and University of California, Davis, Davis, CA.

Body: Introduction: National guidelines recommend annual mammography for surveillance to detect second breast cancer events for all women with a personal history of breast cancer in the absence of new signs or symptoms. Increasingly, women are also receiving surveillance breast magnetic resonance imaging (MRI), despite limited evidence regarding its effectiveness in this population. Our goal was to evaluate performance of surveillance breast MRI and mammography in women with a personal history of breast cancer.

Methods: Study population included 13,038 women aged ≥ 18 with history of stage 0-III breast cancer diagnosed from 2003–2012 who received 33,601 surveillance mammograms and 2,397 surveillance breast MRI examinations from 2005-2012 in the Breast Cancer Surveillance Consortium. Exams were linked to pathology and cancer registry data to assess second breast cancer events within 12 months of the surveillance exam. Descriptive statistics evaluated patient and tumor characteristics associated with use of breast MRI. Unadjusted performance measures were calculated with 95% confidence intervals.

Results: From 2005 to 2012, 11% of women received a breast MRI, and the proportion of use of breast MRI doubled from 4.6% in 2005-2006 to 9.7% in 2011-2012 of women receiving surveillance breast MRI. Patient characteristics associated with breast MRI receipt included: younger age at diagnosis, primary diagnosis of invasive lobular carcinoma, mammographically occult breast cancer, higher AJCC stage and treatment with radiation or chemotherapy, and having heterogeneously or extremely dense breasts. 427 breast cancers were diagnosed within 1 year of a surveillance exam (301 invasive, 126 DCIS; cancer rate 17.9 for breast MRI vs 11.1 mammography per 1000 exams). Cancer detection rates (95%CI) were higher for MRI (10.8; 9.6-12.1) than mammography (7.1; 6.8-7.4) for all cancers and for invasive only (7.5; 6.5-8.6 and 4.7; 4.4-4.9, respectively). Mammography sensitivity was 64.2% (95%CI 59.3-69.1%) and 60.5% (95%CI 45.9-75.1%) for breast MRI (OR=0.94, 95%CI 0.15-5.9=87). Mammography sensitivity for invasive cancer was 59.3% (95% 53.4-65.3%) and was 62.1% for breast MRI (95% CI 44.4-79.7%). Mammography specificity was 93.2% (95% 92.9-93.5%) and 87.8% for breast MRI (95% CI 86.5-89.1%). Biopsy rates at one year per 1000 exams were 3.9 (3.7-4.2) for mammography and 10.1 (95%CI 8.9-11.3) for breast MRI. Positive predictive value among women with biopsy performed was 32.0% (95% CI 28.4-35.5%) for mammography and 21.2% (95%CI 13.7 -28.8%) for breast MRI.

Conclusions: We found significantly higher cancer detection rates (overall and invasive only) among women who received surveillance MRI compared to surveillance mammography alone. Higher cancer detection rates resulted from higher biopsy rates, lower PPV₃, and lower specificity than mammography in women who received surveillance MRI. Further work is needed to account for differences in women who receive MRI and additional research should evaluate the relative performance accounting for confounding due to patient and tumor characteristics, and benefits of MRI for subgroups of women.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-01-02

Title: Trends in positive predictive values following transition from screen film to digital mammography

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Body: Purpose

Our health region converted from screen-film mammography (SFM) to digital mammography (DM) in 2005. DM has several advantages over SFM, including superior contrast resolution, less noise, and opportunities for image optimization through post-acquisition processing; although, the spatial resolution of DM is inferior to SFM. We sought to determine what effect this transition may have had on positive predictive values (PPVs) for malignant and premalignant lesions.

Methods

From our institution's breast biopsy database, we retrospectively reviewed core biopsy results for mammographic calcifications performed in the years 2001-2004 (SFM years) and 2009-2012 (DM years). We subsequently determined the PPV³ (detection of malignancy after biopsy) for each group of years (SFM and DM). We then performed subgroup analysis to calculate PPVs for each of ductal carcinoma in-situ (DCIS) without invasion, DCIS with invasion, and premalignant lesions. Premalignant lesions included atypical ductal hyperplasia, atypical lobular hyperplasia, and lobular carcinoma in-situ.

Results

A total of 4787 biopsies in 4633 patients were reviewed. The comparative detection of cancer after biopsy performed for mammographic calcification between SFM and DM was not statistically significant (PPV³ = 23.5% and 24.0%, respectively; P=.71). Upon further analysis, however, PPV for premalignant lesions increased (SFM=6.6% and DM=8.9%; P<.01) and PPV for DCIS without invasion increased (SFM=15.5% and DM=18.2%; P=.015), while PPV for DCIS with invasion decreased (SFM=8.0% and DM=5.8%; P<.01).

Conclusion

We observed no significant impact on PPV₃ for calcifications following the transition from SFM to DM; however, our subgroup analysis suggests that with digital mammography we are now detecting a statistically significantly lower proportion of DCIS with invasion but greater proportions of DCIS without invasion and premalignant lesions. As the natural history of these lower-grade lesions, particularly in the premalignant category, is still not entirely understood, the significance of potentially detecting more of these earlier cancers/precancers is uncertain.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-01-03

Title: Milky Way sign: A potential predictive sign of breast cancer on digital breast tomosynthesis

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Body: Purpose: “Milky Way sign (MWS)”, which we recently reported¹⁾, is a new finding on digital breast tomosynthesis (DBT) showing microcalcifications overlying non-calcified band-like density. Our study purpose was to describe frequencies of and imaging findings associated with MWS, and to examine the predictive value of MWS for breast cancer.

Materials and Methods: We reviewed all stereotactic core biopsies of suspicious calcifications at our institution from 1/1/2015 to 12/31/2015, finding 124 lesions with calcifications, including 20 malignancies (2 IDC, 5 IDC+DCIS, 13 DCIS) and 104 non-malignant lesions (23 high-risk [14 ADH, 6 LCIS/ALH, 1 intraductal papilloma, 2 other atypical], 81 benign), in 116 patients undergoing both 2D mammogram and DBT before biopsies. 2 radiologists reviewed images for the presence of MWS, local breast density within 1 cm surrounding the calcifications, classifying BI-RADS calcification morphology and distribution. We assessed the predictive value of MWS for malignancy using Chi square test and multivariate logistic analysis.

Results: MWS was identified more frequently with DBT (27/124, 22%) than with 2D (13/124, 10%), and more in locally less dense tissue than in locally dense tissue. The calcifications in MWS were fine pleomorphic (13/27, 48%), amorphous (8/27, 30%), fine linear/branching (5/27, 19%), or other (1/27, 4%), with distributions of grouped (20/27, 74%), linear (5/27, 19%) or segmental (2/27, 7%) categories. MWS on DBT was observed in 60% (12/20, including 2 IDC, 2 IDC+DCIS, 8 DCIS) of malignant lesions and 14% (15/104) of benign lesions (table1), and was significantly and positively associated with malignant lesions ($p < .001$). Multivariate analysis demonstrated the MWS on DBT ($p < .001$) and fine linear/branching calcifications ($p < .001$) were independent predictors for malignancy.

Frequencies of MWS on DBT according to histopathology

	n	MWS positive	MWS negative
Malignancy	20	12 (60)	8 (40)
-IDC	2	2 (100)	0 (0)
-IDC+DCIS	5	2 (40)	3 (60)
-DCIS	13	8 (62)	5 (38)
Non-malignant lesion	104	15 (14)	89 (86)
-ADH	14	3 (21)	11 (79)
-LCIS/ALH	6	0 (0)	6 (100)
-Intraductal papilloma	1	0 (0)	1 (100)
-Other high-risk	2	0 (0)	2 (100)
-Benign	81	12 (15)	69 (85)
Total	124	27 (22)	97 (78)

Data are shown as number with percentage in parenthesis.

Conclusions: Although sample size was limited, our results indicate that DBT may contribute in detecting MWS, and that MWS may be a predictive sign for breast cancers, leading to biopsy of suspicious calcifications.

Reference 1) Xu Y, Miyake KK, Liu YI, Downey JR, Lipson J, Allison K, Ikeda DM. “The Milky Way Sign: a new diagnostic findings of ductal carcinoma on digital tomosynthesis.” The Breast Journal. 2016 May; 22 (3):349-51.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-01-04

Title: Potential clinical application of mammography conducted immediately after contrast enhanced CT as contrast enhanced subtraction mammography

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Body: Background

Contrast Enhanced Subtraction Mammography (CESM) has gained worldwide popularity. It is said that CESM can not only identify tumors but also delineate high-density lesions of the mammary gland that are difficult to be identified by normal mammography (MMG), as well as intraductal progress, and tumor hemodynamics.

In general, imaging is commenced two minutes after injection of the contrast medium, and is conducted in two directions, i.e., left to right craniocaudal view and mediolateral oblique view in the similar manner to the normal MMG.

Subjects and purpose

The study was performed upon the approval of the institutional review board, and included 70 patients who had undergone CESM during the nine-month period from August 2015 to April 2016 using the method that MMG was performed within 2-8 minutes after injection of the contrast medium during contrast enhanced computed tomography. The scan was performed as postoperative follow-up in 29 patients, and as preoperative testing in 41 patients. This method allowed us to obtain additional data with contrast imaging without placing financial burden on the patient, and we investigated whether this method was effective as CESM in the present study.

Results

Of the 41 patients who had undergone the scan as a preoperative test (bilateral scans in 3 patients, and 44 breasts), it was in 43 breasts (97.7 %) that lesions could be identified by MMG, and in 42 breasts (95.5 %) that lesions could be identified by CESM. Of the 44 breasts, three breasts showed high-density mammary glands, in two of which the mass was lateral to the margin of the mammary gland, and therefore could be identified by normal MMG; however, in one breast the mass was medial to the margin of the mammary gland and difficult to be identified by MMG, and could only be identified by CESM. The lesions in the two breasts that were difficult to be identified by CESM exhibited the histological type of DCIS, and the lesions were able to be identified as calcification with normal MMG.

The tumor diameter was compared using invasion size determined by CESM, contrast-enhanced MRI, and postoperative pathological specimen, respectively. The correlation coefficient for CESM and contrast-enhanced MRI was 0.9668, and the correlation coefficient for CESM and postoperative pathological specimen was 0.984887, with a strong correlation observed (Pearson's correlation coefficient test).

Conclusion

To the best of our knowledge, no reports of CESM in which MMG is performed immediately after contrast-enhanced CT have been published to date, and thus this is the world's first attempt of such method performed at our hospital. CESM ensures diagnosability without being invasive or placing financial burden on the patient. It was suggested that CESM can be clinically useful particularly for those who cannot undergo contrast-enhanced MRI due to hardware problems, financial difficulties, or metal embedded within the body.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-01-05

Title: Influence of hormone replacement therapy following bilateral salpingo-oophorectomy on mammographic breast density in women newly diagnosed with breast cancer

Pivo S, Schwartz S, Chun J, Guth A, Axelrod D, Shapiro R and Schnabel F. New York University Langone Medical Center, School of Medicine, New York, NY and New York University Langone Medical Center, New York, NY.

Body: Background: Studies have demonstrated that use of hormone replacement therapy (HRT) in post-menopausal women results in increased breast density. This is associated with increased risk of breast cancer and reduced sensitivity of mammography. The purpose of the present study was to compare breast densities of women following surgical menopause with and without use of HRT to women who had natural menopause without use of HRT.

Methods: Our institutional Database was queried for post-menopausal women newly diagnosed with breast cancer from 01/2010 to 01/2016. Patients were divided into following groups: 1) natural menopause with no HRT use, 2) history of a bilateral salpingo-oophorectomy (BSO) with no HRT use, 3) history of a BSO with HRT use prior to diagnosis, or 4) history of BSO and use of HRT at the time of diagnosis. BSO may have occurred with or without concurrent hysterectomy.

Results: 1106 women were eligible for analysis. 976 (88%) had natural menopause with no HRT, 63 (6%) had a BSO with no HRT, 51 (5%) had a BSO with previous history of HRT, and 16 (1%) had a BSO and were using HRT at time of malignancy diagnosis. Though not statistically significant, women who had a prior BSO and were on HRT at the time of diagnosis had more dense breasts (69% heterogeneously or extremely dense) than women who had natural menopause with no HRT (44% heterogeneously or extremely dense). However, women who had a prior BSO and were on HRT in the past had significantly less dense breasts ($p=0.007$) than women who underwent natural menopause without HRT use (67% vs. 56%).

Breast densities by group

Variables	Natural menopause, no HRT (N=976, 88%)	BSO, no HRT (N=63, 6%)	p-value	BSO, prior HRT (N=51, 5%)	p-value	BSO, current HRT (N=16, 1%)	p-value
Median age at diagnosis, yr	64 (39-95)	64 (39-93)	0.48	65 (45-83)	0.34	62 (41-77)	0.17
Median age at menopause, yr	51 (33-62)	47 (32-60)	<0.0001	46 (30-59)	<0.0001	47 (39-55)	<0.0001
Breast density							
Entirely fatty	94 (10%)	5 (8%)	0.95	0 (0%)	0.007	0 (0%)	0.17
Scattered fibroglandular	448 (46%)	30 (47%)		34 (67%)		5 (31%)	
Heterogeneously dense	393 (40%)	25 (40%)		16 (31%)		11 (69%)	
Extremely dense	41 (4%)	3 (5%)		1 (2%)		0 (0%)	

There were no differences observed in the tumor characteristics between all groups.

Conclusion: In our study cohort, women with history of surgical menopause who were on HRT at the time of breast cancer diagnosis had a trend towards more dense breasts. Surprisingly, women who were prior users had less dense breasts than women who never used HRT. Despite the change observed in breast density, the tumor characteristics do not differ with HRT usage, suggesting that variability in breast density did not correlate with stage of disease at presentation. This may be related to heightened awareness among clinicians regarding risk associated with HRT use that led them to modify screening and surveillance in these patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-01-06

Title: Visualizing the real difference between 2-D and 3-D specimen mammography

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Body: BACKGROUND: Specimen mammography aids in the determination 1) if the target lesion has been removed and 2) whether there is a clear margin at excision. In the past, two orthogonal views using 2-D imaging has been considered to be equivalent to a three dimensional perspective. Yet tomosynthesis for screening mammography has demonstrated the value of thin sliced imaging over two view screening mammography. In March, 2015, we began using true 3-D tomosynthesis of breast specimens at lumpectomy and have compared 2-D and 3-D specimen mammography.

METHODS: We have examined 125 consecutive breast cancer patients with both 2-D and 3-D imaging of the same specimens since March 2015. The circulating nurse would take the specimen and obtain two orthogonal views using both 2-D and 3-D devices with images sent to the radiology department. It was not felt ethical to blind the surgeon from having both images available to make an intraoperative clinical decision regarding immediate re-excision. We compared the data noted from each method and which method best aided the decision to perform immediate re-excision, and time required to obtain the images.

RESULTS: We have studied 125 patients over 11 months since March 2015. Confirmation of complete lesion excision was easier with 3-D tomosynthesis than with 2-D as the 3-D slice did not include overlying skin or dense breast tissue surrounding the lesion in the image, making the target lesion stand out more clearly. Although the 2-D images appear to have higher contrast than the individual 3-D slices, the tomosynthesis 3-D images contained more actionable data than the 2-D. Also decisions to excise more tissue during the procedure were enabled by the additional information included in the 3-D images. In addition, the 3-D images provided the depth of field to enable accurate re-excision using the Z-axis (see figures). Finally, it took about a minute longer to obtain and review the 3D images, although this difference did not delay surgical decisions nor prolong operative time.

CONCLUSIONS: 3-D specimen tomosynthesis facilitates the reduction of post-operative re-excision for lumpectomy patients by providing more information than 2-D orthogonal views, providing easier, more accurate confirmation of the extent of the target excision. Additionally, serial 1mm slices of the specimen allowed the integration of Z-axis targeting, ensuring that any necessary margin excision during surgery was accomplished immediately with maximum tissue conservation. More studies are planned to further validate these findings of these first 125 patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-01-07

Title: BI-RADS classification in daily practice: Keep it simple

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Body: Aim

To investigate the risk of malignancy following stereotactic biopsy of calcifications classified as BI-RADS 3, 4 and 5.

Methods

All women with calcifications (not associated with masses or architectural distortions) who underwent stereotactic breast biopsy procedures at the Dutch Cancer Institute between January 2011 and October 2013 were included in the study. Suspicious calcifications (defined as BI-RADS 3, 4 or 5) detected on mammography were biopsied. All lesions were analyzed by dedicated breast radiologists and classified according to the BI-RADS lexicon.

Findings

Overall, 473 patients underwent 497 stereotactic breast biopsy procedures. Sixty-six percent (326/497) of the calcifications were classified as B4, 30% (148/497) were classified as B3 and 4% (23/497) were classified as B5. In total, 226 (45%) lesions were malignant. The malignant lesions consisted of 182 cases of pure DCIS (ductal carcinoma in situ), 22 cases of mixed DCIS and invasive carcinoma (ductal or lobular), 21 cases of pure invasive carcinomas (ductal or lobular) and 1 angiosarcoma. Malignancy was found in 32% (47/148) of the B3, 49% (160/326) of the B4 and 83% (19/23) of the B5 calcifications.

Conclusion

Considering the high incidence of malignancy in B3 calcifications, we believe that these lesions should either be classified as clearly benign (B2) or suspicious (B4 or 5) to avoid missing a malignant lesion. This simplification could improve patient care, avoiding delays in both diagnosis and treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-02-01

Title: Pre-operative breast MRI would not benefit breast cancer patients' survival, even in young patients treated with breast-conservative surgery

Huang N, Chen J, Yang B, Quan C, Xue J, Huang X and Wu J. Fudan University Shanghai Cancer Center, Shanghai, China and Shanghai Medical College, Fudan University, Shanghai, China.

Body: Background

Although breast magnetic resonance imaging (MRI) could help to identify occult lesions in breast cancer, its role in patient outcome has always been controversial. The current study aimed to evaluate the role of MRI in Asian breast cancer patients, especially young patients that might have dense breasts.

Methods

Patients with non-metastatic unilateral breast cancer who received surgery in our institute during 2007-2013 were retrospectively reviewed. The differences between groups were compared using Pearson's χ^2 test. Loco-regional recurrence-free survival (LRRFS) and recurrence-free survival (RFS) were estimated using the Kaplan-Meier method.

Results

A total of 13,681 patients were included into analysis, among which 5823 (42.6%) had pre-operative MRI. Of all patients, 39.7% were stage 0-I according to TNM system, 35.5% were stage II, 14.8% were stage III and 10.0% could not be staged. Patients with axillary lymph node metastasis were comparable in MRI and non-MRI groups (35.3% versus 35.6%, $P=0.695$). The percentile of patients receiving MRI increased from 7.3% in 2007 to 67.5% in 2013. Patients in the breast-conservative surgery (BCS) group were more likely to receive MRI compared with the mastectomy group ($P<0.001$).

Age and menopausal status were also related with the choice of pre-operative MRI. When patients were grouped according to age, 56.7%, 48.9%, 43.6%, 37.0% and 27.1% patients in the ≤ 35 , 36-45, 46-55, 56-65 and >65 years old group had MRI, respectively ($P<0.001$). When patients were grouped according to menopausal status, 37.8%, 48.8% and 27.3% patients in the post-menopausal group, pre-menopausal group and unknown menopausal status group had MRI, respectively ($P<0.001$).

In survival analysis, the average follow-up time for the MRI group ($N=5823$) and non MRI group ($N=7858$) were 88.5 and 114.4 months, during which 238 (4.1%) and 464 (5.9%) breast cancer recurrences occurred, while 63 (1.1%) and 159 (2.0%) loco-regional recurrences occurred. The estimated 5-year RFS for MRI group and non-MRI group were 90.1% and 90.0% ($P=0.510$). The 5-year LRR-free survival LRRFS for MRI group and non-MRI group were 96.7% and 97.3% ($P=0.128$). In subgroup analysis, 2376 patients received BCS, and 11,035 received mastectomy. Patient in the pre-operative MRI group did not have superior RFS or LRRFS compared with the non-MRI group, regardless of surgical management (BCS versus mastectomy). Then we restrained the analysis to patients who were ≤ 45 years old and treated with BCS. A total of 699 patients had pre-operative MRI, and 419 patients did not. The 5-year RFS for MRI group and non-MRI group were 95.8% versus 94.6% ($P=0.231$); the 5-year LRRFS for MRI group and non-MRI group were 99.8% and 97.1% ($P=0.144$).

Conclusions

There was an increasing trend of pre-operative MRI examination during 2007-2013, especially in young patients and patients treated with BCS. However, pre-operative MRI could not benefit breast cancer patients' survival by detecting occult lesions, even in young patients treated with BCS, who were considered to have dense breasts.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-02-02

Title: Predictive value of breast MRI in detecting mammographically occult contralateral breast cancer: Can we target women more likely to have contralateral breast cancer based on primary tumor clinicopathologic factors?

Susnik B, Lillemoe TJ J, Swenson KK K, Tsai ML L, Finkelstein MJ J, Schneider L, Braatz CM M, Krueger JL L and Rueth N. Allina Health, Virginia Piper Cancer Institute, Minneapolis, MN and Allina Health, Piper Breast Center, Minneapolis, MN.

Body: Background: Use of preoperative magnetic resonance imaging (MRI) staging in newly diagnosed breast cancer increases detection of synchronous contralateral breast cancer (CBC) over other screening modalities; however, it is associated with a high false positive rate, additional biopsies, extensive surgical procedures, and possibly increased psychological morbidity.

Specific Aims: To determine predictors of synchronous, mammographically-occult but MRI-detected CBC in women newly diagnosed with breast cancer.

Methods: We performed a retrospective review of patients at Allina Health, Abbott Northwestern Hospital who had preoperative breast MRI prior to surgical resection of their breast cancer from 2010–2014. We collected patient demographic and clinicopathologic data. To determine the association between MRI-detected CBC versus benign findings based on clinicopathologic data, we performed univariate analysis ($p < 0.05$). Multivariate Logistic Regression was used to adjust for covariates and factors predictive of CBC. Area under the Receiver Operating Characteristic Curve provided a measure of model accuracy.

Findings: 1894 patients had pre-operative MRI during the study period. Of those, 201 had suspicious findings on contralateral breast MRI requiring biopsy (table 1). Overall 3% (60/1894) had synchronous CBC (invasive carcinoma or DCIS) detected on MRI. The majority of CBCs ($n=60$) were stage 0 or IA (85%), ER/PgR+ (98%), HER2- (89%), and low/intermediate grade (80%). Women more likely to have mammographically-occult CBC were older ($p < 0.001$), had lobular versus ductal index cancer ($p=0.03$), and had ER positive ($p=0.027$) or PgR positive ($p=0.002$) tumors. On multivariate logistic regression analysis (ROC area=0.75), PgR positive status ($p=0.022$), and older age ($p=0.004$) were predictive of CBC. With each year of additional age, odds of CBC increased by 5%. No CBC was identified in women $<$ age 45 with high risk index cancers (ER- or HER2 +). CBC was 11 times more likely when PgR status was positive versus negative. CBCs were diagnosed significantly more frequently in patients with index cancers that were hormone receptor positive and HER2 negative compared to HER2 positive or triple negative invasive index cancers (Fisher's exact test; $p=0.041$).

Risk of CBC by Index Cancer Type

Index Cancer	CBC n (%)
DCIS (n=51)	15 (29)
Invasive carcinoma (n=150)	45 (30)
ER+/HER2- (n=121)	41 (34)
HER2 positive (n=18)	3 (17)
Triple Negative (n=11)	1 (9)
Total (n=201)	60 (30)

Conclusions: Preoperative MRI is effective in detecting mammographically-occult early stage, hormone receptor positive CBC in older women. MRI-detected CBC is more common in patients originally presenting with hormone receptor positive and HER2 negative index cancers. Our results suggest that it may be possible to determine a subset of patients who are less likely to benefit from a preoperative breast MRI, an important implication in an era of increasing healthcare cost utilization awareness.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-02-03

Title: Accurate and reliable automated breast density measurements with no ionizing radiation using fat-water decomposition MRI

Ding J, Thompson PA A, Gao Y, Marron MT T, Wertheim BC C, Altbach MI I, Galons J-P, Roe DJ J, Wang F, Maskarinec G, Thomson CA A, Stopeck A and Huang C. Stony Brook University, Stony Brook, NY; Stony Brook Medicine, Stony Brook, NY; University of Arizona, Tucson, AZ and University of Hawaii at Manoa, Honolulu, HI.

Body: Objective Breast density(BD) is a measure of the distribution of variable tissue types within the breast and higher BD has been shown to positively correlate with breast cancer risk. As such, the accurate measurement of BD has become a priority for risk assessment and for evaluating the effects of prevention strategies aimed at reducing BD. Mammography(MG) is the most common method of BD determination but is limited by the exposure to ionizing radiation, particularly for studies requiring repeated measures. BD derived from fat-water decomposition magnetic resonance imaging(FWMRI-BD) has been proposed as an alternative, safe, and quantitative method for BD. To optimize its use, we developed a new FWMRI-BD that is automated, more accurate and reliable. In this study, we compare our automated method to digital MG and a previous reported algorithm for MRI derived BD.

Methods From a completed prevention trial, 42 pre- and post-menopausal patients receiving tamoxifen therapy for early stage breast cancer or as primary chemoprevention were identified. Patients had undergone prior digital MG within 6 months from the date of MRI scan and MG-BD was calculated using a well-established method(Cumulus). MRI scans were performed on a 1.5T GE Signa NV-CV/i scanner using an axial radial IDEAL-GRASE sequence to generate quantitative fat fraction maps of the entire breast. Total acquisition time was < 5 min and automated breast segmentation was applied to all scans. Only the contralateral, unaffected breast was analyzed. Pearson correlation analysis compared BD as measured by MG(range 0-100%) and FWMRI based methods. BD by FWMRI was initially calculated as the ratio of breast voxels with<80% apparent fat fraction(Fra_{80}). Fra_{80} had been previously shown by our group to correlate with MG-BD(Spearman $\rho=0.86$, $p<0.001$). Here, BD was calculated using a new algorithm(Fra_{G+W}) that accounts for the total amount of fibroglandular tissue and water content in the breast after correction for fat-water signal intensity bias and fat-water signal shine-through. Reliability of FWMRI measurements was tested in 24 repeated scans from 9 patients and evaluated using intra-class correlation(ICC) analysis.

Results Table 1 shows the correlation and reliability analysis results between MG-BD and FWMRI-BD. Both FWMRI-BD measures(Fra_{80} and Fra_{G+W}) were strongly correlated with MG-BD. More importantly, they exhibit superior test-retest reliability(ICC>0.98) compared to MG-BD values from the literature(reported ICC range 0.91-0.95). Fra_{G+W} showed improvement over Fra_{80} in all measures tested including correlation to MG-BD, dynamic range, standard errors and ICC.

Table 1. Accuracy and Reliability of the FWMRI-BD measures

FWMRI-BD	Fra80	FraG+W
Pearson correlation coefficient* with MG-BD	R=0.86	R=0.94
Test-retest reliability		
standard error	0.0230	0.0134
dynamic range	0.0902 – 0.6537	0.0736 – 0.6588
standard error/ dynamic range	4.1%	2.3%
ICC [95% confidence interval]	0.985 [0.966,0.993]	0.990 [0.976,0.995]

* All P-values < 1e-10

Conclusion The refined and automated FWMRI-BD that quantifies the entire fibroglandular and water content of the breast(Fra_{G+W}) strongly correlates with MG-BD and is more accurate and reliable than previous FWMRI-BD method.

Acknowledgement NIH grants CA149417, CA161534.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-02-04

Title: Is breast magnetic resonance imaging (MRI) useful for diagnosis of additional sites of disease in patients recently diagnosed with ductal carcinoma in situ (DCIS)?

Ortiz-Perez T, Benveniste AP P, Ebuoma LO O, Sepulveda KA A, Severs FJ J, Kapoor M and Sedgwick EL L. Baylor College of Medicine, Houston, TX.

Body: Purpose: To determine if breast MRI is useful for detecting additional sites of disease in patients initially diagnosed with pure DCIS.

Materials and Methods: A retrospective review of women diagnosed with pure DCIS who underwent a breast MRI for evaluation of extent of disease was performed at a single institution from January 2013 to April 2015. Data analysis included imaging (mammography, ultrasound and MRI) and pathology characteristics (histology and biomarker status) of the primary DCIS as well as descriptors for the additional sites of disease incidentally found by breast MRI.

Results: A total of 94 patients were diagnosed with pure DCIS during this time period, ages ranging from 38 to 79 years old (median, 58.5 years); sizes ranging from 0.6 to 16 cm (mean, 4.5 cm). A total of 28 patients (30%) had other MRI findings suspicious for additional sites of disease in either breast. From this group of patients, 23 (82%) patients underwent MRI-guided biopsy. The 5 patients who did not have the MRI guided-biopsy either underwent total mastectomies or declined biopsy. Five out of the twenty-three patients (22%) were diagnosed with an additional site of cancer. Three of these patients were Hispanic, one was Asian/Pacific and one was Caucasian. Of the five patients, five had contralateral disease (100%) and none had a second site of disease in the ipsilateral breast. The size of the additional sites of disease ranged from 0.4 to 7.2 cm (mean, 2.1 cm) and the size of the primary lesion in this selected group ranged from 0.4 to 9 cm (mean, 3.4 cm). Ages ranged from 47 to 63 years old (median, 55 years). Four out of five patients (80%) presented with the first site of disease as pure DCIS with estrogen (ER) and progesterone (PR) receptors positive and one case (20%) was pure DCIS ER/PR- negative. The second incidental lesion found on MRI demonstrated 3 cases of contralateral pure DCIS. From this group, all the biomarkers status for the first and second site were concordant. From the 5 cases of second disease, 2 (40%) presented with invasive component in the contralateral side of the initially biopsy-proven pure DCIS and, one of these cases had discordant biomarkers compared with the first site of disease, the first site of pure DCIS was ER/PR-negative and the second site of invasive ductal carcinoma (IDC) presented with ER/PR-positive status.

Conclusion: From a total of 94 patients with recent diagnosis of pure DCIS who underwent breast MRI examination for diagnosis of additional sites of disease, approximately 5% were diagnosed with an additional site of cancer and 2% of the total cases had invasive disease in the additional sites with different biomarker status; changing their management and prognosis. Breast MRI plays a role in the setting of staging patients initially diagnosed with pure DCIS.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-02-05

Title: Does MRI influence surgical planning more than clinical outcome? A cohort study of breast cancer patients receiving neoadjuvant therapy

McDermott M, Wilson C, Xu J, Illmann C and Simmons C. University of Waterloo, Waterloo, ON, Canada; BC Cancer Agency, Vancouver, BC, Canada and University of British Columbia, Vancouver, BC, Canada.

Body: Background: While magnetic resonance imaging (MRI) is a powerful diagnostic tool, there is currently no consensus on its role for breast cancer patients prior to the initiation of neoadjuvant therapy (NAT). In the adjuvant setting, there is evidence that the use of MRI is correlated with an increase the rate of mastectomies performed. There is currently no data describing how MRI is influencing treatment decisions or surgical management in the neoadjuvant setting. This study aimed to determine the impact of MRI on patients' surgical plan, and to understand the demographic differences in patients who had an MRI compared to those that did not in the neoadjuvant setting.

Methods: A secure database containing all potential NAT patients seen by medical oncologists at the BC Cancer Agency Vancouver Centre since 2012 was searched. Breast cancer patients who were treated with NAT and had undergone breast surgery before March 30, 2016 were identified. Tumour characteristics, surgical plan and surgical outcome were assessed retrospectively and compared between patients who had an MRI and patients who did not have an MRI.

Results: 270 patients were identified who met the inclusion criteria. Of those, 107 patients had a breast MRI and 163 patients did not. The two groups showed no significant pre-treatment differences with regards to type of breast cancer, receptor status, or clinical stage. The median age was 10 years younger in the MRI group (47 years) compared to the non-MRI group (57 years), $p < 0.0001$. Patients who had an MRI had a non-significant higher rate of pathological complete response (pCR) than those who did not (30.8% and 21.5%, respectively, $p=0.08$). The surgical treatment did differ between these two groups; those who had MRI were more likely to have bilateral mastectomy (36.4% vs 23.3%, $p=0.019$) and less likely to have breast conserving surgery (BCS) (19.6% vs 31.9%, $p=0.026$). In the cohort that had an MRI, there was no significant difference in percentage of patients whose surgical plan was changed compared to the patients who did not have an MRI (33.6% and 28.8%, respectively). A change in surgical plan from a mastectomy to a BCS was more common in patients who did not have an MRI than those that did (31.9% and 13.9%, respectively). 45% of the surgeons who dictated a follow-up surgery consultation stated that the MRI was used to inform the surgical plan.

Discussions/Conclusions: In this real-world cohort, patients who had an MRI were more likely to undergo a bilateral mastectomy and less likely to have a BCS than the patients who did not have an MRI, despite having a higher rate of pCR. Age was the only baseline demographic difference between the two groups. These findings suggest that the role of MRI in the neoadjuvant setting needs to be refined further in order to avoid over-treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-02-06

Title: Magnetic resonance imaging (MRI) surveillance for patients with dense breasts and a previous breast cancer (BC) and/or high risk lesion

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Body: BACKGROUND AND PURPOSE

The benefits of breast MRI for screening women at high risk of developing BC is established, but its role in women with a personal history of BC or dense breasts is unknown. We sought to estimate the performance of annual surveillance MRI added to mammography in women at moderately increased BC risk due to a personal history of breast cancer and/or a high-risk breast lesion and dense breasts.

METHOD AND MATERIALS

We performed a retrospective chart review of the clinical, radiological, and pathological parameters of women who received annual, concurrent surveillance breast MRI and mammography between 04/2013 and 12/2015. We included women who met all of the following criteria: age < 69; prior diagnosis of high-risk lesion (ADH, ALH, LCIS), DCIS, or invasive BC; heterogeneously (50-75%) or extremely dense (>75%) breasts; and did not qualify for our provincial MRI screening program for high risk women (calculated lifetime BC risk \geq 25%). Results of each scan were analyzed using descriptive statistics and Chi squared for comparisons between subgroups.

RESULTS

A total of 199 patients (267 MRI exams) were included in this study. The mean age at initial diagnosis was 45 years and at subsequent diagnosis of DCIS or invasive cancer was 53 years. Mean time to new diagnosis was 86 months (range 14-202). All 15 cancers diagnosed during the study period were MRI detected: 11 invasive stage I (66% IDC, 7% ILC) and 4 DCIS (27%). Of these 15, all but 1 were mammographically occult. Five (33%) were found in the breast ipsilateral to the original lesion. The cancer detection rate was 6% (12/199) on the first screening round and 4.7% (3/64) on the second screening round. Specificity and positive predictive value respectively for MRI exams increased from 77% and 22% on the first screening round to 88% and 30% on the second round. Of women who developed BC, 57% had a history of breast or ovarian cancer in a first degree relative. None of the 72 women who were on hormonal therapy at the time of surveillance imaging had a new cancer detected compared to 11% (14/125) of those who were not on hormonal therapy ($p=0.0025$).

CONCLUSIONS

The incremental early-stage BC detection rate and specificity of MRI in this population are comparable to what is observed in screening women at high risk. The addition of annual MRI to mammography should be considered for surveillance of women with a personal history of BC / premalignant lesion and heterogeneous / extremely dense breasts, particularly if they have a family history of BC and are not on hormonal therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-02-07

Title: Predictors associated with MRI surveillance screening in women with a personal history of unilateral breast cancer but without a genetic predisposition for future contralateral breast cancer

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Body: Purpose

Women with a personal history of breast cancer (BC), in the absence of genetic predisposition or significant family history, are generally categorized as having an intermediate (15-20%) risk of developing future BC. Screening MRI is recommended as a supplement to mammography for women with a $\geq 20\%$ lifetime risk. However, models available to calculate risk exclude women with a personal history of BC. Thus, there has existed no reliable mechanism to calculate future risk in these women to refine MRI surveillance recommendations. The Manchester score was recently developed and intended to inform contralateral breast cancer (CBC) risk for surgical decision making. We hypothesized that the score could be informative to guide follow-up imaging recommendations among women with a personal history of BC.

Patients & Methods

322 women with newly-diagnosed, non-metastatic, unilateral BC were seen in multidisciplinary breast clinic and underwent unilateral surgery (either breast conserving surgery or mastectomy) at our institution between June 2012 and November 2015. Using life expectancy, family history, genetic mutation status, and endocrine therapy use, we calculated the Manchester score, i.e. CBC risk, for all women. Patients were categorized as low- (<10% CBC lifetime risk), above average- (10-20%), moderate- (20-30%), and high-risk (>30%). We also reviewed the rationale that treating physicians noted for recommending MRI surveillance. Univariate logistic regression analysis (UVA) was used to assess if Manchester score was predictive of MRI surveillance in addition to other known factors.

Results

In the entire cohort, 75.8% (n=244) were low-risk for CBC, 18.6% (n=60) were above average-risk, 4.3% (n=14) were moderate-risk, and 1.2% (n=4) were high-risk. Using a 20% CBC risk as a threshold for MRI justification, 5.6% (n=18) met indications for MRI surveillance. Among the 21.7% (n=70) undergoing MRI surveillance, 57.1% (n=40) were low-risk, 32.9% (n=23) were above average-risk, 7.1% (n=5) were moderate-risk, and 2.9% (n=2) were high-risk. There was a significant trend for higher rates of MRI surveillance as the risk score increased (odds ratio, OR 1.1, $p < 0.0001$). On UVA, the highest odds for MRI surveillance were in women with a mammographically-occult BC history (OR 9.35, $p < 0.0001$), pre-operative breast MRI use (OR 8.41, $p < 0.0001$), and dense breast tissue (OR 4.88, $p < 0.0001$). The top clinician-endorsed reasons for MRI surveillance were dense breast tissue (61.4%), young age at diagnosis (28.6%), and mammographically-occult BC history (25.7%).

Conclusions

Although Manchester score was significantly predictive for MRI surveillance, we identified a large subset of women with <20% calculated CBC risk who underwent MRI surveillance (90.0% of the cohort undergoing MRI) and a small subset with $\geq 20\%$ CBC risk who continue with mammography alone (4.4%). Overall, concern about poor detection of a future CBC appeared to dominate the selection of surveillance MRI use, even within a largely low-risk population. We believe this calculation could be informative prospectively to select surveillance strategies in women at a high future risk of CBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-02-08

Title: Association of background parenchymal enhancement with breast cancer risk factors and tumor characteristics

Iyer L, Faramand R, Jung S, Rosenblatt P, Campassi C, Dromi S, Dorgan J and Tkaczuk K. University of Maryland Greenbaum Comprehensive Cancer Center, Baltimore, MD.

Body: Background: Background Parenchymal Enhancement (BPE) in a dynamic contrast enhanced (DCE)MRI refers to change in morphology and temporal degree of enhancement expressing physiology of breast tissue. Improvements in the analysis and interpretation of BPE have been shown to increase diagnostic accuracy of the MRI. We explored associations of BPE with breast cancer(BC) risk factors and tumor characteristics.

Methods: We conducted a retrospective chart review study of 149 women who were treated for BC at the University of Maryland Medical Center(UMMC) between 2003-2015. Institutional Review Board approval was obtained. Subjects had BI-RADS 4,5 or 6 on their mammograms and/or biopsy proven BC. Women with false positive mammograms (n=2), missing BPE grade in their MRI reports (n=6), metastatic BC at diagnosis (n=2), MRIs done outside of a year of diagnosis (n=3), and who followed up outside UMMC (n=1) were excluded. Final analysis included 135 women. We used 1.5T or 3T scanners for DCE MRI. Standard contrast enhanced MRI protocol, T1, T2, and dynamic series were acquired according to American College of Radiology requirements for breast MRI accreditation and interpreted with Maximum Intensity Projection and subtracted imaging. Fellowship-trained breast imagers performed qualitative BPE assessment as per standard BI-RADS classification. BI-RADS classes were grouped as high BPE (BI-RADS moderate and marked) and low BPE (BI-RADS minimal and mild). Multivariable logistic regression was used to assess associations with predictors including individual and tumor characteristics.

Results: Preliminary analyses showed BPE was significantly associated with alcohol intake, with drinkers being more likely to have high BPE compared to non-drinkers [odds ratio (OR) =3.08 (95% confidence interval (CI) = 1.34-7.09; p=0.008]. Women who received adjuvant radiation for their BC were less likely to have high BPE compared to women who did not undergo radiation [OR= 0.37 (95% CI=0.15-0.90); p=0.03] implying higher likelihood of breast conservation surgery in the former group. High BPE was less often observed for invasive ductal carcinoma histology vs. in-situ ductal carcinomas [OR= 0.40 95% CI=0.16-1.00]; p=0.05] and in post- vs. premenopausal women [OR=0.47 (95%CI=0.22-1.00); p=0.05]. No association was found between BPE and race, BMI, prior HRT/OCP use, smoking, or ER/PR/HER-2 status.

Conclusion: We observed association between higher BPE and alcohol exposure and lower BPE and, invasive ductal histology, postmenopausal status at diagnosis of BC and adjuvant radiation for BC. Larger studies are needed to corroborate our findings and identify the underlying mechanisms.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-02-09

Title: Do radiographic features influence the decision to order a breast MRI? A prospective cohort study

Illmann C, Simmons CE E, McDermott M, Xu J and Wilson C. BC Cancer Agency, Vancouver, BC, Canada.

Body: Background:

The clinical impact of breast MRI in the neoadjuvant setting is unclear. It is assumed that MRI may help with surgical planning. Factors that may affect whether an MRI is ordered for breast cancer in the neoadjuvant setting may include both imaging and tumour characteristics. Literature suggests MRI can be used in the neoadjuvant therapy (NAT) if there is evidence of high density of breast tissue, multifocal disease, multi-centric disease, lymph node involvement or presence of calcifications. In a non-trial setting, it is unclear when MRI is ordered and if it is indeed ordered based on the above imaging criteria. We sought to determine how MRI is currently implemented in a provincial practice to determine which patients are selected for MRI prior to NAT. Specifically, we aimed to determine if the imaging characteristics determined likelihood of use of MRI in the neoadjuvant setting.

Methods:

Patients who received neoadjuvant therapy between May 2012 and May 2016 were captured in a prospective database at the BC Cancer Agency in Vancouver. Patients were reviewed and identified as those who either received a breast MRI or not. A random sample of 80 cases, 40 who received MRI and 40 who did not, was taken from this database. Charts were reviewed in detail, and detailed review of the radiographic features from mammogram and ultrasound imaging reports was recorded.

Results:

80 patients were reviewed in detail. There were no differences in patient demographics or tumour characteristics. Imaging review demonstrated no statistical significant difference in use of MRI based on reported density, multi-centric disease, calcifications, and nodal involvement. The only radiographic feature that was different was presence of multifocal disease on conventional imaging, where 40.0% of patients who had an MRI had multifocal disease reported whereas only 17.5% of those who did not have an MRI had multifocality reported ($p = 0.03$).

Discussion/Conclusions:

Despite radiographic guidelines for use of MRI, the decision by the ordering physician regarding who should receive an MRI prior to NAT still appears to be unsystematic. This could be due to incorrect interpretation of radiographic reports by the ordering physicians and lack of availability or access to the interpreting radiologist. Results suggest that the ordering physician is already aware of multifocal disease and is utilizing MRI to verify this presence, rather than using MRI to investigate the possibility of multifocality in dense breast tissue. Based on this strategy of use it is unlikely that MRI will reduce the rate of mastectomy in this patient population.

Table 1: Summary of radiographic features in MRI vs. non-MRI cohorts

	MRI (N=40)	No MRI (N=40)	Chi Square
Density (C or D)	25 (62.5%)	16 (40.0%)	$p=0.11$
Multifocal Disease	16 (40.0%)	7 (17.5%)	$p=0.03$
Multi-centric Disease	7 (17.5%)	3 (7.5%)	$p=0.19$
Lymph Node Involvement	31 (77.5%)	25 (62.5%)	$p=0.25$
Calcifications Present	23 (57.5%)	21 (52.5%)	$p=0.56$

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-02-10

Title: Selective use of MRI and impact on management in breast cancer

Jafferbhoy S, Tandon M, Kirby R, Narayanan S, Bajwa S, Salehi Bird S, Mohd-Isa Z and Soumian S. Royal Stoke University Hospital, Stoke-on-Trent, Staffordshire, United Kingdom.

Body: Introduction:

Breast cancer can present as a mammographically occult lesion. Lobular carcinoma has the propensity to develop multifocal and multicentric disease. Studies have suggested that invasive carcinoma with lobular features have similar biological characteristics as invasive lobular carcinoma. In our unit, patients with mammographically occult cancer and those with lobular features on core biopsy have a pre-operative MRI scan. The aim of this study is to assess the impact of contrast enhanced MRI on management of these cases.

Methods:

Over a 3 year period from November 2012 to October 2015, all breast MRI scans were reviewed and those patients with mammographically occult cancer and invasive carcinoma with lobular features on biopsy were included. Demographic data, imaging, pathology and treatment details were collected from Clinical Information System. A size difference of +/-10% between the imaging modalities was considered to be concordant

Results:

Out of 389 patients with breast MRI for invasive carcinoma, 104 patients with a median age of 57 years were included. 69 patients (66%) were symptomatic and 35 (34%) were screen-detected cancers. 64 patients had lobular features on core biopsy and 40 patients had mammographically occult cancer.

In patients with lobular features group, MRI findings were concordant with mammograms in 26 patients (40%) while 38 (60%) had additional findings (28% multifocality, 19% non-concordant size, 6% contralateral findings and 7% ipsilateral benign findings).

In patients with non-concordant findings, 58% underwent USS and 24% had biopsy following MRI.

In the mammographically occult group, USS identified the primary lesion in 32 patients (80%) while 8 patients (20%) had occult lesion both on USS and mammograms. MRI findings were concordant in 90% while 10% had non-concordant findings (6% multifocality and 4% non-concordant size). Following MRI, 8% had another USS and 3% had biopsy.

MRI findings changed the treatment plan in 19% cases with lobular features. 14% underwent mastectomy instead of wide local excision, 3% primary chemotherapy and 2% bilateral wide local excision. There was a change in treatment plan in 25% of the mammographically occult cancers of which only 6% had lesion visible on USS. 15% of mammographically occult cancers had mastectomy instead of WLE and 10% had neo-adjuvant chemotherapy.

Conclusion:

This study has demonstrated that pre-operative MRI leads to additional investigations. Its impact on management is low if the lesion is mammographically occult but visible on ultrasound. In cases with ultrasound occult cancer and those with lobular features on core biopsy, it changes management in a significant proportion and should therefore be considered as a part of the diagnostic work-up.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-02-11

Title: Clinical examination and breast MRI as predictors of pathologic complete response post neoadjuvant therapy in HER2 overexpressed subtypes and triple negative breast cancer

Andrade FEM Martins, De Barros ACS Simao, Docema MF Fernando, Heinzen RN Neves, De Andrade JZ Zabukas, Nimir C, Mano MDS De Senna, Gianotti D and Ribeiro KDCB Braga. Hospital Sírio Libanês (HSL), São Paulo, Brazil.

Body: Background: Preoperative identification of pathologic complete response (pCR) is important to decrease surgical morbidity. The objective of this study was to determine diagnostic validity of clinical examination and magnetic resonance imaging (MRI) in determining pathologic response in patients with breast cancer subtypes HER 2 overexpressed and triple negative after neoadjuvant therapy.

Methods: This is a cross-sectional study, with a sample comprising 72 patients woman with HER-2 overexpressed or triple negative breast submitted to neoadjuvant treatment at Hospital Sirio Libanês between January 2005 and December 2012. All patients were clinically evaluated by a group of seven breast surgeons. Double reading of breast MRI was performed in three periods: at the beginning of treatment, after the second cycle of chemotherapy and after treatment. Photographic record of the breast was done before and after chemotherapy. HER-2 and hormone receptors were assessed using immunohistochemistry. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (VNP) were estimated using pathology as the gold standard. Area under ROC curve and the corresponding 95% confidence intervals (95% CI) were calculated.

Results: Thirty-two patients (44,4%) had triple negative tumors while 40 (55.6%) overexpressed HER-2. Among those with triple negative tumors, clinical examination evidenced a completed response in 31.2% (10/32) of the cases. pCR was observed in 3 patients (9.4%). Diagnostic validity measures for clinical examination were: Se= 100%, Sp=75.9%, PPV=42.9% and NPV=100%. In this group, MRI detected a complete response in 7 cases (21.9%). Therefore, MRI presented a Se=66.8%, Sp=82.8%, PPV 28.6% and NPV= 96%. Area under ROC curve was 0.88 (95% CI 0.80-0.96) and 0.75 (95% ci 0.41-1.00) for clinical examination and MRI, respectively. Among woman with tumors over expressing HER-2, complete response was observed through clinical examination in 45% (18/40) of these cases, showing Se= 100%, Sp=71%, PPV=100% and NPV=70,9%. In this group, complete radiological response was noted in 8 cases (20%). Therefor, MRI had Se=33.3%, Sp=83.9%, PPV 37.5% and NPV=96%. Area under ROC curve was 0.85 (95% CI 0.77-0.94) and 0.59 (95% CI 0.41-0.76) for clinical examination and MRI, respectively.

Conclusions: Our findings demonstrate that clinical examination is superior to MRI to predict pCR for woman wth tumor overexpressing HER-2, while for tumor patients with triple negative tumors the two methods were equivalent. Therefore, clinical examination can be used with MRI to monitor tumor response to neoadjuvant chemotherapy and also to determine the best course of surgical action. Monitoring and assessment, however, are better when both methods are associates.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-02-12

Title: Impact of magnetic resonance imaging on conversion from wide local excision to mastectomy in patients with ductal carcinoma in situ: First results from the ECOG-ACRIN 4112 prospective study

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Body: Purpose: To estimate the proportion of patients with ductal carcinoma in situ (DCIS) judged to be candidates for wide local excision (WLE) based on mammography (+/- sonography) and clinical exam who: (A) convert from WLE to mastectomy as the first surgical procedure based on magnetic resonance imaging (MRI) findings, and (B) have a mastectomy as the final surgical procedure.

Patients and Methods: The study population included women with DCIS diagnosed by core needle biopsy (and no evidence of invasive or micro-invasive disease) judged to be candidates for WLE by their surgeon based on standard imaging (mammogram +/-sonography) and clinical exam without prior breast MRI. After registration bilateral contrast enhanced breast MRI was performed, and a protocol management algorithm was followed. Before and after MRI, the surgeon documented the planned surgical procedure, including reason for conversion from WLE to mastectomy if the surgical plan changed. Prior evidence suggests that about 8-12% of patients initially considered candidates for WLE based on mammography ultimately require mastectomy, and the addition of MRI increases the likelihood of mastectomy by about 1.5-fold. A sample size of 350 (332 evaluable) was planned to ensure a 95% confidence interval (C.I.) of 8% if the actual mastectomy conversion rate was as high as 16%. Two-sided 95% Wilson confidence interval for proportion of patients converting to mastectomy based on MRI was derived.

Results: Of 363 patients enrolled, 340 had sufficient information to assess aim A (conversion to mastectomy after MRI), and 322 for aim B (mastectomy as final surgical procedure). At study entry, median age (range) was 59 years (34-87). Features of DCIS lesions included: median longest diameter (range) 11 mm (1-100); ER positivity 76.2%; low, intermediate and high grade 15.9%, 40.9% and 39.4% respectively (3.8% unknown). Based on MRI findings (Aim A), mastectomy rather than WLE was recommended as the first surgical procedure in 25/340 (7.4%, 95% CI: 5.0%, 10.6%). Reasons included large lesion size (N=15), multi-centric disease (N=6), and contralateral disease prompting bilateral mastectomy (N=4). An additional 28/340 (8.2%) converted to mastectomy as the first surgery based on other factors, including patient preference (N=25), interval discovery of genetic mutation (N=2), or inability to receive radiation treatment (N=1). Of the 278 patients for whom initial WLE was performed after MRI, 10/278 (3.7%) converted to mastectomy, all due to positive or close (< 2 mm) surgical margins. Overall (Aim B), 62/322 (19.3%, 95% CI 15.3%, 23.9%) had mastectomy as the final surgical procedure.

Conclusion: In women with DCIS judged to be candidates for WLE based on mammography (+/- sonography), conversion to mastectomy is fairly common with nearly 1 in 5 patients ultimately undergoing mastectomy. Breast MRI findings account for less than half of the conversions to mastectomy. For patients who remained candidates for WLE after MRI, 96.3% achieve successful WLE as the final surgical procedure.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-01

Title: A novel high-throughput secreted factor screen and bioinformatics pipeline identifies microenvironment-derived FGF2 as a mechanism of resistance to anti-estrogens, PI3K, and mTOR inhibitors in ER+ breast cancer

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Body: Resistance to anti-estrogen therapy is a serious obstacle to the treatment of patients with ER+ breast cancer. Unlike the majority of preclinical studies that focus on cancer cell-intrinsic mechanisms of resistance, we hypothesized that the tumor microenvironment significantly contributes to drug resistance, and that individual factors present in the microenvironment differentially modulate response to therapy.

To systematically test this hypothesis, high-throughput screens were performed in which 297 unique secreted proteins (cytokines, growth factors, and extracellular matrix) were tested on ER+ breast cancer cell lines (MCF-7 and T47D) treated +/- anti-estrogen fulvestrant or the PI3K inhibitor GDC-0941 (pictilisib). Cytokines that rescued cells (hits) were validated in two additional ER+ breast cancer cell lines (ZR75-1 and HCC-1500), and expanded to include the anti-estrogen tamoxifen and the mTORC1 inhibitor everolimus. Hits were also found to rescue from combinations of fulvestrant/GDC-0941 and fulvestrant/everolimus in models with acquired resistance to fulvestrant. Multiple factors, such as ErbB family ligands and fibroblast growth factors (FGFs), were among the top validated hits.

To parse out which hits are most likely to be relevant in the setting of the ER+ breast tumor microenvironment, a bioinformatics filter was developed to incorporate gene and protein expression data for secreted proteins in non-cancer human tissues relevant to ER+ breast cancer. These tissues include primary breast tissue components (breast mammary, adipose, primary fibroblasts) and common metastatic sites (bone marrow, lung, liver). After filtering, the top hit was fibroblast growth factor 2 (FGF2), which significantly drives resistance to anti-estrogens, PI3K, and mTOR inhibitors, and is highly expressed in non-cancer tissues relevant to ER+ breast cancer.

FGF2-mediated rescue was completely abrogated by the ATP-competitive pan-FGFR inhibitor PD173074, confirming FGFR kinase specificity of the rescue phenotype. Immunoblot data confirmed that FGF2 induced phosphorylation of FGFR and the FGFR effector FRS2, and a consistent pattern of downstream MEK-ERK-Rsk90 activation for rescue to all drugs. Flow cytometry data showed that FGF2-mediated rescue leads to decreases in drug-induced apoptosis and G1 cell cycle arrest, which correspond at the protein level to increased degradation of the pro-apoptotic protein Bim and upregulation of the cell cycle driver cyclin D1, respectively.

Mice bearing MCF-7 xenografts were treated with vehicle, FGF2 (20 ug/kg/d, s.c.), fulvestrant (5 mg/wk, s.c.), or the combination. Vehicle-treated and FGF2-treated mice showed similar rates of tumor growth. Single-agent fulvestrant significantly suppressed tumor growth, while the addition of FGF2 rescued from the growth-inhibitory effect of fulvestrant. Studies in other tumor models are ongoing. These data collectively suggest that stroma-derived FGF2-mediated drug resistance is a novel therapeutic opportunity in ER+ breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-02

Title: Inhibition of NOS promotes ER stress response and augments docetaxel-mediated apoptosis in TNBC

Davila-Gonzalez D, Choi DS, Kuhn J, Granados SM M, Rosato RR R, Dave B and Chang JC C. Methodist Cancer Center, Houston Methodist Hospital, Houston, TX; Tecnologico de Monterrey, Campus Monterrey, Monterrey, Nuevo Leon, Mexico; UT College of Pharmacy, UTHSCSA, San Antonio, TX; Hospital Complex of Jaen, Jaen, Spain and GENYO, Centre for Genomics and Oncological Research, Granada, Spain.

Body: Introduction: Chemoresistance in triple negative breast cancer (TNBC) is related to an activation of a survival response orchestrated by endoplasmic reticulum (ER) stress. We hypothesize that attenuation of nitric oxide (NO) signaling pathway can overcome treatment resistance, preventing relapse, ultimately improving survival of TNBC patients. Here, we aimed to investigate the effects of pharmacological iNOS (inducible nitric oxide synthase) inhibition by L-NMMA on docetaxel-mediated ER stress response and to determine whether the therapeutic NOS inhibition may improve chemotherapy-based response.

Methods: BT-549, SUM-149, MDA-MB-436, and MDA-MD-468 TNBC cell lines were treated with docetaxel (D; 5 nm)/ L-NMMA (L; 4mM)/ amlodipine (A; 5 µM) daily for 48 and 72 hours. Cell death and proliferation were assayed by Annexin V and ATP quantification, respectively. Western Blot (WB) was used to measure ER stress markers. *In vivo* regimen treatment followed three 2-weekscycles of D (20 mg/kg intraperitoneal [IP] on day 1) and L (200 mg/kg oral gavage on day 2-6); A (10 mg/kg IP on day 2-6) A was administered together with L to counteract the well-known effects of L on blood pressure (hypertension). TNBC Patient derived xenograft (PDX) models #2147, #5998, #3107 and #4664 were transplanted into the mammary fat pad of SCID Beige mice. PDX #2147 received either, single drug (vehicle, L, A, D), double (L+A, D+L, D+A), or triple drug combination (L+A+D). Models #4664, #3107 and #5998 received only vehicle, D or D+ L+A. Mice weight and tumor volumes were recorded twice weekly. D concentration was measured by mass spectrometry.

Results: Studies on SUM-159 cell line showed that, when compared to the docetaxel-treated group, D+L+A increased cell death significantly, as indicated by a rise in annexin V/propidium iodide-positive cells. Increase in cell death by D+L+A was further demonstrated by accumulation of mitochondrial cleaved BAX. The enhanced apoptotic effects of D+L+A in MDA MD 468, BT 549 and MDA MD TNBC cell lines were confirmed by a decrease in ATP levels compared to D alone. WB revealed a survival stress response activated by docetaxel. When it was coupled with NOS inhibition, ER stress response showed higher expression of ATF4 and CHOP, triggering a proapoptotic response by pASK1/JNK pathway and cleaved caspases (CC3 and CC9). PDX #2147 showed that L, A and L+A treatment groups had similar tumor volume growth as the untreated group. However, combination therapy, D+L+A, significantly reduced the tumor volume and increased survival proportions compared with vehicle and docetaxel. Combination therapy also dramatically reduced tumor size on TNBC #4664 and #3107, and significantly improved response on #5998 compared with docetaxel alone. Intratumoral docetaxel concentration was 5.3-fold higher in mice receiving D+L+A than in those receiving docetaxel alone (#5998). In both groups, docetaxel was not detected in the plasma one week after injection.

Conclusion: The present data suggest that iNOS may be a critical target for docetaxel resistance in TNBC. iNOS inhibition enhanced chemotherapy response in TNBC PDX models indicating that addition of iNOS inhibitor may improve prognosis and prevent relapse in TNBC patients who have failed conventional chemotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-03

Title: An acquired HER2 T798I gatekeeper mutation induces resistance to neratinib in a patient with HER2 mutant-driven breast cancer

Hanker AB B, Red Brewer M, Sheehan JH H, Koch JP P, Lanman R, Hyman DM M, Cutler, Jr. RE E, Lalani AS S, Cross D, Lovly CM M, Meiler J and Arteaga CL L. Vanderbilt University Medical Center, Nashville, TN; Guardant Health, Redwood City, CA; Memorial Sloan Kettering Cancer Center, New York, NY; Puma Biotechnology, Inc., Los Angeles, CA and Astra Zeneca, Cambridge, United Kingdom.

Body: *ERBB2*, the gene encoding HER2, is mutated in 2-4% of breast cancers. The HER2 irreversible tyrosine kinase inhibitor (TKI) neratinib has shown clinical activity against breast cancer cells harboring HER2 activating mutations. Here, we report for the first time an acquired gatekeeper HER2^{T798I} mutation in a patient with HER2-mutant breast cancer after an initial exceptional response to neratinib.

A patient with ER+/PR+/HER2-negative invasive lobular breast cancer progressing on standard therapy was found to harbor a L869R kinase domain mutation in HER2. HER2^{L869R} is homologous to the known activating mutation EGFR^{L861R/Q}. MCF10A breast epithelial cells expressing HER2^{L869R} displayed enhanced HER2-mediated signaling and were resistant to lapatinib and trastuzumab but sensitive to neratinib. The patient was enrolled in the phase II SUMMIT trial (NCT01953926) and treated with neratinib, achieving a partial response lasting 16 months before developing progression. Next gen sequencing of DNA from both a new skin metastasis and plasma cell-free DNA (cfDNA) identified HER2^{L869R} (8.7% cfDNA), whereas a novel HER2^{T798I} mutation was detected only in plasma at 1.3%. Deep sequencing of pre-therapy tumor tissue and plasma did not detect HER2^{T798I}, suggesting that this mutation arose upon resistance. HER2^{T798I} has not been reported in TCGA, COSMIC, or among plasma samples from 17,345 cancer patients subjected to digital DNA sequencing using the Guardant360 assay.

HER2^{T798I} is homologous to the EGFR^{T790M}, KIT^{T670I} and BCR-ABL^{T315I} gatekeeper mutations known to mediate resistance to erlotinib/gefitinib and imatinib. To examine if HER2^{T798I} mediates resistance to neratinib, we employed biochemical and biological assays and molecular modeling of wild-type (WT) HER2 and HER2^{T798I}. Structural modeling showed the increased bulk of the isoleucine at position 798 would result in a steric clash with neratinib, thus reducing drug binding. We stably expressed HER2^{WT}, HER2^{T798I}, HER2^{L869R} and HER2^{L869R/T798I} in MCF10A cells and NR6 mouse fibroblasts. Neratinib (10-100 nM) blocked HER2-mediated signaling in cells expressing HER2^{WT} or HER2^{L869R} but did not in cells expressing HER2^{T798I}. The EGFR irreversible TKI osimertinib (100 nM), which is selective for mutant EGFR (including EGFR^{T790M}) and approved for treatment of NSCLC expressing EGFR^{T790M}, failed to inhibit HER2^{WT}, HER2^{L869R} or HER2^{T798I}. In contrast, either the EGFR/HER2 irreversible TKI afatinib or AZ5104, a metabolite of osimertinib, strongly blocked signaling induced by HER2^{WT}, HER2^{L869R} or HER2^{T798I}. Cells expressing HER2^{T798I} displayed a significantly higher IC₅₀ to neratinib than cells expressing HER2^{WT}, whereas afatinib or AZ5104 were very active against all cells (IC₅₀<10 nM).

Conclusions: The acquisition of a T798I gatekeeper mutation in HER2 upon development of clinical resistance to neratinib in a breast cancer with an initial activating mutation in HER2 strongly suggests that HER2^{L869R} is a driver mutation. We speculate that HER2^{T798I} may arise as a secondary mutation following response to effective HER2 TKIs in other cancers with HER2 activating mutations. Certain irreversible EGFR inhibitors may be effective in patients with HER2-driven breast cancer resistant to neratinib.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-04

Title: Sensitivity to lapatinib differs between HER2-amplified breast cancer cells harboring kinase and helical domain mutations in *PIK3CA* and relies on production of PIP₃

Garay JP P, Korkola JE E and Gray JW W. Oregon Health & Science University, Portland, OR.

Body: Introduction: HER2 is amplified in nearly 25% of all primary breast cancers. Lapatinib is a targeted therapy that inhibits overactive HER2 signaling but invariably resistance to this targeted therapy occurs in a substantial number of patients. The PI3K-AKT axis is the major pathway downstream of HER2 signaling. Activated PI3-kinase phosphorylates the membrane lipid PIP₂ resulting in PIP₃. PIP₃ is as a docking site for pleckstrin homology (PH) domain proteins, such as the AKT. AKT influences a variety of pathways inside the cell involving cell growth, regulation of apoptosis, glucose metabolism, and others. Mutations in the gene *PIK3CA* deregulate this signaling axis. In HER2 amplified cancers, co-occurrence of *PIK3CA* mutations have been reported in approximately 20% of cases. Hotspot mutations of *PIK3CA* translate to changes in either the helical domain (E545K) or kinase domain (H1047R) of the protein and these two hotspots comprise over 80% of all reported oncogenic mutations across all tumor types. Crystallographic studies have shown conformational differences between the two hotspot mutations in *PIK3CA*, yet it is unclear if functional differences exist between the two mutations.

Methods: We generated isogenic knockin mutants of the helical domain (E545K) and kinase domain (H1047R) of *PIK3CA* in the HER2-amplified breast cancer cell line SK-BR-3. Mutant and parental cell lines were subjected to drug sensitivity assays measured by cell growth during prolonged exposure to drug. We investigated changes of relevant intracellular signaling pathways via western blot analysis. Additionally, we used immunofluorescence of PIP₃ and confocal microscopy to visualize cellular differences in the production of this signaling molecule.

Results: Our results demonstrate a distinction between the helical domain (E545K) and kinase domain (H1047R) mutations of *PIK3CA*. Mutations in the helical domain do not confer resistance to lapatinib while mutations in the kinase domain do. This is a result of sustained AKT signaling even in the presence of high dose lapatinib in cells with the kinase domain mutation. We also show the PTEN loss phenocopies this phenomenon. Finally, we show that kinase domain mutations allow the protein to generate significantly higher levels of PIP₃ which is the necessary molecule for downstream signaling through AKT but helical domain mutations do not.

Conclusion: This phenotypic disparity between helical and kinase domain mutations of *PIK3CA* has important clinical implications. It is possible to imagine that in a heterogeneous tumor in which some cells are wildtype and some cells carry this mutation for *PIK3CA* treatment with lapatinib will select for cells with the mutation conferring a growth advantage. Our results show that only H1047R mutant cells demonstrate lapatinib resistance and this is achieved via sustained AKT signaling through continual production of PIP₃. Altogether, we demonstrate a mechanism of *de novo* resistance to HER2-targeted therapy in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-05

Title: PI3K/PDK1 mediates resistance to CDK4/6 inhibitors through dysregulation of S-phase cyclins/cyclin dependent kinases (CDKs)

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Vanderbilt University Medical Center, Nashville, TN; UT Southwestern Medical Center, Dallas, TX and University of Arizona, Tucson, AZ.

Body: Background: CDK4/6 inhibitors in combination with antiestrogens are approved for the treatment of ER+ advanced breast cancer. However, not all patients benefit from CDK4/6 inhibitors, underscoring the need to develop therapeutic strategies to circumvent *de novo* and acquired drug resistance.

Methods: ER+ breast cancer cells (MCF-7, T47D, HCC1428, and HCC1500) were made resistant to increasing doses to the CDK4/6 inhibitor ribociclib (LEE011; Novartis). LEE011-resistant cells were characterized by 2D/3D growth, cell cycle, and immunoblot analyses. GSK2334470 (PDK1 inhibitor) and dinaciclib (CDK2 inhibitor) were used to modify resistance to ribociclib. PDK1 and pS6 immunohistochemistry (IHC) were performed on primary human tumor explants treated *ex vivo* with palbociclib.

Results: Resistant cell lines (MCF-7/LR, T47D/LR, HCC1428/LR, and HCC1500/LR) exhibited an IC₅₀ at least 20-fold higher than that of their parental cells. They displayed cross-resistance to the CDK4/6 inhibitors palbociclib and abemaciclib. Immunoblot analysis of ribociclib-resistant cells showed increased levels of 3-phosphoinositide dependent protein kinase 1 (PDK1), S227 pRSK2 (target of PDK1), T308 pAKT (target of PDK1), and pS6 (downstream effector of the PDK1 target p70S6K), compared to parental drug sensitive cells. PDK1 is a master kinase that functions downstream of phosphoinositide 3-kinase (PI3K) and is crucial for the activation of AKT and many other AGC kinases including PKC, S6K, SGK, and RSK. Primary tumor explants treated *ex vivo* with palbociclib for 96 h also exhibited upregulation of PDK1 and pS6 by IHC. Cell cycle analysis revealed that CDK4/6 inhibition failed to induce G1 arrest, a reduction in S phase, and senescence in MCF-7/LR and T47D/LR compared to parental cells. Progression into S phase in the presence of ribociclib suggested upregulation of S-phase cyclins/CDKs. Indeed, the resistant cells exhibited significantly higher levels of pCDK2, cyclin A, cyclin E and S477/T479 pAKT, a CDK2-dependent phosphorylation of AKT required for full kinase activity and limited to the S-phase of the cell cycle. Pharmacological inhibition of PDK1 (with GSK2334470) or CDK2 (with dinaciclib) re-sensitized the ribociclib-resistant cells to CDK4/6 inhibitors. However, ribociclib/GSK2334470 inhibited MCF-7/LR and T47D/LR cell proliferation better than ribociclib/dinaciclib. Further, ribociclib/GSK2334470 but not ribociclib/dinaciclib completely abrogated pRb, pS6, pRSK2, pCDK2, cyclin A, and cyclin E, suggesting the PI3K/PDK1 pathway mediates acquired resistance to CDK4/6 inhibitors through dysregulation of the cell cycle. Consistent with these data, ribociclib/GSK2334470 inhibited growth of established MCF-7 xenografts in nude mice, significantly more potently than each drug alone.

Conclusions: These data support a critical role for PI3K/PDK1 in acquired resistance to CDK4/6 inhibitors in ER+ breast cancer cells. Co-targeting of PI3K/PDK1 and CDK4/6 may overcome resistance to CDK4/6 inhibitors and is worthy of further translational and clinical investigation in patients with ER+ breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-06

Title: Characterization of HER2-positive breast cancer (BC) cells selected for tolerance to trastuzumab-induced antibody-dependent cell-mediated cytotoxicity (ADCC)

Biswas T, Fritzscheier R, Mark A, Meißner T, Young B, Jones BL L and Pegram M. Stanford Cancer Institute, Stanford School of Medicine, Stanford, CA; University of Washington, Seattle, WA; Avera Cancer Institute, La Jolla, CA and Avera Medical Group Precision Oncology, Sioux Falls, SD.

Body: Cellular mechanisms of trastuzumab resistance include alteration(s) in cell signaling pathways (PTEN loss, activation of PI3K/Akt signaling), steric hindrance of antibody binding (by Muc-1/Muc-3), over-activation of alternate receptor kinases (HER3/c-Met/IGF-1R), and proteolysis of HER2 extracellular domain harboring target epitopes for antibody-based therapeutics. Prior studies of trastuzumab resistance have focused largely on cells selected *ex vivo* with the antibody in absence of human immune effector cells. We developed a selection model, wherein human HER2 positive BC cells (BT474, SKBR3) were subjected to acute ADCC (>90% cell death), trastuzumab concentration 100ug/mL, effector-target ratio 100:1, using human peripheral blood mononuclear cells (PBMCs) as effectors. Surviving cells were allowed to recover to confluence over 8-10 weeks, for 10 total rounds of ADCC selection *ex vivo*. Mock-treated parent, IgG1 isotype control, trastuzumab, and PBMCs alone were used as controls. ADCC assays based on calcein fluorescent labeling of live target cells, revealed significant reduction (maximum 20%, $p < 0.005$) in cell lysis in immune-selected BT474 cell lines compared to parental controls (immune-selected SKBR3 cells exhibited a non-significant trend towards reduced ADCC). Transcriptome-wide next-generation RNA sequencing (Illumina NextSeq 500, 2 x 75 bp paired-end, median of 46 million paired-end reads/sample), coupled with pathway enrichment analysis (Reactome), followed by q-PCR validation, confirmed significant changes in expression in immune-selected cells (compared to parent control) for genes including: ALDH1, ANK1, TMPRSS3, HINT1, DNMT2, TNNC1, COL4A4 in BT474; and ALDH1, ANK1, CAMP1, CPE, IDO1 in SKBR3 cells. Whole-genome sequencing (Illumina HiSeq X, 150 bp paired end, 30x coverage) elucidated 180 genes with single nucleotide variations (SNVs) in immune-selected cells compared to parent in BT474 cells, and 215 genes in SKBR3 cells. Thirty-four SNVs were shared in both cell lines. Further screening and validation confirm genes with SNVs demonstrating significant transcript up-regulation. These include: COL4A3, LEP, SOX-9 in BT474; and HLA-B, TNFRSF10B, HLA-B, PSMA6 in SKBR3. In further phenotypic analysis, ADCC-conditioned BT474 cells exhibit an elongated fibroblast-like morphology with multiple processes, in contrast to control. Immune-selected SKBR3 cells (and not BT474 cells) demonstrate significantly increased motility compared to control in transwell migration assays ($p \leq 0.001$), and demonstrated increased cell proliferation (MTT assay, 10-15%, 48h; $p = 0.0242$) as compared to parent controls. Our data indicate immune-selection by effector cells contributes to ADCC tolerance *in vitro*, and is associated with distinct genotypic and phenotypic alterations. Future investigation will determine whether Fc-engineered MAbs (afucosylated), antibody drug conjugates (T-DM1), or potentiation of ADCC by co-stimulatory agonist CD137 antibodies will re-sensitize ADCC-tolerance. This investigation will help to elucidate potentially targetable pathways that emerge from immune-selection with trastuzumab.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-07

Title: High-throughput pharmacogenomic platform to functionally probe breast cancer vulnerabilities in the context of the 3-dimensional tumor microenvironment

Dhimolea E, De Matos Simoes R, Awate P, Tang H, Culhane A and Mitsiades C. Dana Farber Cancer Institute, Boston, MA.

Body: The pharmacological vulnerabilities of malignant cells have been traditionally evaluated by the use of cell lines grown in vitro in 2-dimensional (2D) plastic surfaces. In contrast, malignant tumors in vivo grow as 3-dimensional (3D) cellular masses. We used Estrogen Receptor (ER)-positive (MCF7, ZR75-1 and T47D) and triple-negative (MDA-MB-231) breast cancer (BrCa) cells to assess the differences between the malignant growth in 2D vs. 3D conditions with regards to morphology, molecular characteristics and response to therapeutic agents. The ER-positive BrCa cells formed compact spheroids when grown in collagen type I or matrigel; in contrast the MDA-MB-231 cells assumed an invasive phenotype characterized by infiltrating bundles of cells. Antiestrogens induced acinar differentiation in the compact spheroid morphology of ER-positive BrCa cells. This effect of antiestrogens was blocked in co-cultures of BrCa cells with bone marrow stromal cells, suggesting that the 3-dimensional microenvironment of distant metastatic sites can affect the susceptibility of BrCa cells to anti-tumor agents. We further probed this question through pharmacological screens of ~100 FDA-approved antineoplastic agents and 400 target-annotated kinase inhibitors, to assess the pathophysiological relevance of these in vitro interactions and to identify specific BrCa vulnerabilities in 3D conditions. The 3D spheroid morphology was associated with significant decrease in the efficacy of several classes of conventional DNA-damaging (e.g. anthracyclins) and anti-microtubule (e.g. vinca alkaloids) agents, proteasome inhibitors, as well as recently established targeted therapies, including kinase inhibitors. This differential 3D spheroid-associated drug resistance vs. sensitization was more pronounced for the triple-negative MDA-MB-231 cell line compared to the ER-positive BrCa cells. Interestingly, inhibition of folate metabolism induced reduced viability of ER-positive BrCa spheroids, indicating increased dependency of ER-positive tumor cells on this pathway, under 3D growth conditions. Interestingly, mut-p53 T47D 3D spheroids were resistant to antifolates, suggesting a possible role of functional p53 for the anti-BrCa effect of antifolates in 3D conditions. Gene expression analysis and reverse-phase protein array analyses of BrCa cells in this system indicated that growth of malignant cells as 3D spheroids was associated with switch to glycolysis metabolism and up-regulation of survival signals (e.g. Mcl1, Hsp70 et.c.). We conducted a genome-wide CRISPR screen in MCF7 and MDAMB231 2D cultures and identified several known and previously underappreciated BrCa essential genes; we are currently using the insight obtained by these molecular analysis to conduct focused CRISPR screens in our 3D cultures, to validate the genetic vulnerabilities observed in 2D conditions and to determine the mediators of malignant growth and drug resistance in 3D conditions. This approach can reveal novel and previously underappreciated therapeutic targets for drug-resistant BrCa.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-08

Title: A large-scale functional screen to identify resistance mechanisms to selective estrogen receptor degraders fulvestrant and GDC-810 in ER+ breast cancer

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Body: Therapies that target the estrogen receptor provide clinical benefit and improved survival for patients with estrogen receptor-positive (ER+) breast cancer, yet drug resistance remains a challenging problem, leading to disease relapse and mortality. In recent years, the selective estrogen receptor degrader (SERD) fulvestrant has become an important therapeutic option for patients with resistant ER+ metastatic breast cancer, and newer oral SERDs such as GDC-810 are currently being tested in clinical trials. The mechanisms of intrinsic and acquired resistance to SERDs remain to be fully elucidated. We conducted a large-scale lentiviral open reading frame (ORF) screen to identify genes whose overexpression confers drug resistance to either fulvestrant or GDC-810 in the ER+ breast cancer cell line T47D. The lentiviral ORF expression library used in this study consists of 16,544 barcoded ORFs, including 2,767 ORFs with mutations. The initial screen yielded 72 genes resulting in resistance to fulvestrant and 85 genes resulting in resistance to GDC-810, with 44 genes overlapping. The top ranked-genes included multiple genes belonging to the PI3K/Akt, ERbB/HER, and FGF/FGFR pathways as well as genes involved in cell cycle progression.

Fibroblast growth factor receptor 1 (FGFR1) amplifications are frequently observed in patients with ER+ breast cancer, and have previously been implicated in resistance to endocrine therapies. Several FGFs (FGF3, FGF6, FGF10, and FGF22) were among the top-ranked resistance genes for both fulvestrant and GDC-810, suggesting that activation of the FGFR signaling pathway may render cells resistant to fulvestrant and GDC-810. In the presence of FGF2, overexpression of FGFR1 in ER+ breast cancer cells resulted in resistance to both fulvestrant and GDC-810. The ability of an FGFR inhibitor to overcome FGFR-mediated resistance to SERDs is being tested. Additional potential resistance genes identified in the ORF screen are also being validated. In summary, a whole-genome functional resistance screen has identified several candidate genes and pathways that may cause resistance to fulvestrant and GDC-810. Several of these candidates, such as FGFR1, are also found in patients who develop resistance to SERDs, suggesting rational combination therapies to overcome or preempt SERD resistance.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-09

Title: Resistance to palbociclib depends on multiple targetable mechanisms highlighting the potential of drug holidays and drug switching to improve therapeutic outcome

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Body: Background: Combination of CDK4/6 inhibitors and endocrine therapy has been shown to improve clinical outcome in advanced estrogen receptor-positive (ER+) breast cancer (BC) patients. However, most patients will progress with acquired resistance, making the identification of new therapeutic strategies and potential biomarkers of great importance. Here, we show for the first time that certain resistance mechanisms are amenable to drug holidays and drug switching while others cause irreversible resistance to further perturbation of G1/S and highlight the potential of targeting these tumours by blockade of G2/M with inhibitors of Wee1 or CDK7.

Methods: To identify adaptive mechanisms associated with resistance to palbociclib, MCF7 and T47D cells adapted to long term oestrogen deprivation (LTED), which either retain or lose expression of ER respectively, were grown in the presence of palbociclib until they became resistant (LTED^{991R}). Cell lines were subjected to exome sequencing, global gene expression analysis and siRNA knockdown of 709 cellular kinases to identify candidates associated with resistance. Candidate drug targets were evaluated in proliferation assays. Data was validated *in vivo* using MCF7-LTED xenografts.

Results: Exome data showed few genetic changes were associated with resistance to palbociclib. MCF7-LTED^{991R} had a copy number (CN) gain of *CCNE2* and *ESR1* compared to MCF7-LTED while T47D-LTED^{991R} showed loss of *RB*. Global gene expression analysis revealed increased expression of *CDK4*, *CDK2*, *CDK7* and *CCNE1* in MCF7-LTED^{991R} and *CCNE2* and *CDK2* in T47D-LTED^{991R}. In order to assess if these changes were drug specific, cell lines were treated with another CDK4/6 inhibitor, abemaciclib. Strikingly, MCF7-LTED^{991R} cells showed sensitivity, possibly as a result of increased CDK4 expression, for which abemaciclib has increased potency. We next assessed the plasticity of the 991R phenotype by giving the cells a drug holiday followed by re-challenge. T47D-LTED^{991R} cells remained resistant whilst the MCF7-LTED^{991R} cells were re-sensitised, an observation we confirmed in a MCF7-LTED xenograft after long term treatment with palbociclib. Finally, siRNA kinome knockdown highlighted CDK4, CDK7 and Wee1 as associated with the resistant phenotypes suggesting targeting of G2/M in both *RB+* and *RB-* 991R tumours may provide benefit; this possibility was confirmed by increased sensitivity to THZ1 (CDK7 inhibitor) or MK1775 (Wee1 inhibitor).

Conclusion: Few genetic changes are associated with resistance to palbociclib in ER+ BC *in vitro* but kinase re-wiring provides resistance. *RB* loss of function appears an irreversible mechanism of resistance while gain of cyclin E, and overexpression of CDK2 and CDK4 are amenable to a drug holiday, leading to re-sensitisation to palbociclib *in vitro* and *in vivo*. Of note, the palbociclib-resistant cells that retained *RB* expression were sensitive to abemaciclib possibly as a result of increased expression of CDK4 acting as compensatory mechanism. Finally, LTED cell lines retaining ER that were resistant to palbociclib also showed a gain in *ESR1* CN, highlighting the potential for combination therapy with an alternate endocrine agent, such as fulvestrant.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-10

Title: Penfluridol reduces paclitaxel resistance in breast cancer by inhibiting HER2/ β -catenin signaling

Gupta N, Gupta P and Srivastava S. Texas Tech University Health Sciences Center, Amarillo, TX.

Body: Breast cancer is the most common malignant carcinoma in women worldwide. It has been estimated that each year almost 182,000 cases of breast cancer are diagnosed and over 40,000 women die of breast cancer in the US, making it the second leading cause of cancer related deaths. Metastatic breast cancer is a major cause of mortality. Paclitaxel is a promising therapeutic agent for the treatment of patients with metastatic breast cancer. However, inherited or acquired resistance is a major limiting factor for successful therapy with paclitaxel in many patients. The mechanism of paclitaxel resistance remains obscured and has hindered the development of useful therapeutic strategies. HER2 is an oncogene, overexpressed in about 30% of breast cancer patients and plays role in drug resistance leading to poor prognosis. To identify more clinically relevant mechanism of paclitaxel resistance, we developed paclitaxel resistant MCF-7 and 4T1 breast cancer cell lines. We evaluated the cytotoxicity of paclitaxel in these cell lines to confirm resistance. Furthermore, we characterized the molecular changes in these cells as compared to parent cells using molecular techniques and cell-based assays. The continuous exposure to paclitaxel resulted in >100 fold resistance towards paclitaxel in MCF-7 and 4T1 cells. Western blot analysis showed enhanced HER2 expression in resistant cells. Interestingly, HER2 was substantially localized in the nucleus of resistant cells as compared to parent cells. We also observed enhanced expression of β -catenin in the resistant cells. β -catenin is known to promote cancer progression in HER2 over-expressing breast tumors. Hence we hypothesized that HER2/ β -catenin mediates resistance to paclitaxel and suppression of HER2 and β -catenin signaling could inhibit paclitaxel resistance. In another study, we recently demonstrated that penfluridol suppresses the growth of triple negative metastatic breast cancer cells (*Ranjan and Srivastava, Cancer Res 2016; 76(4):877-890*). We therefore, treated paclitaxel resistant cells with penfluridol to see if resistant cells would be sensitive to penfluridol treatment. Our current results showed that penfluridol treatment not only suppressed the expression of HER2 in paclitaxel resistant cells but also inhibited β -catenin and its downstream effector molecules resulting in reduced survival of paclitaxel-resistant breast cancer cells. Our results further showed that penfluridol treatment synergistically enhanced the growth suppressive effects of paclitaxel. Taken together, our results provide a novel insight into the mechanism of resistance to paclitaxel and also open new avenue for application of penfluridol in cancer therapeutics. Further mechanistic and *in vivo* studies are in progress. It is important to note that penfluridol is an FDA approved anti-psychotic drug with an established safety and efficacy profile. Hence based on our observations, penfluridol can be a fast track agent that can be made available to the patients resistant to paclitaxel in relatively shorter period of time. [Supported in part by R01 grant CA129038 (to S.K.S.) awarded by National Cancer Institute, NIH].

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-11

Title: Tamoxifen-resistant breast cancer cells are resistant to DNA-damaging chemotherapy because of upregulated BARD1 and BRCA1

Liu Q, Zhu Y and Liu Y. Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, Guangdong, China.

Body: Tamoxifen is the most widely used endocrine therapy in estrogen receptor (ER)- positive breast cancer patients. Tamoxifen resistance has been a major clinical problem and is accountable for relapse in about one third of these patients. Most of these recurrent patients will inevitably receive chemotherapy. However, the association between tamoxifen-resistance and chemosensitivity in breast cancer has never been explored. We found that both mRNA and protein expression of BARD1 and BRCA1 were significantly upregulated in tamoxifen-resistant MCF7 and T47D breast cancer cell lines, when compared with their parental lines. Furthermore, the tamoxifen-resistant cells were markedly more resistant to DNA-damaging chemotherapy including cisplatin and doxorubicin, but not to paclitaxel. Silencing BARD1 or BRCA1 by siRNAs or inhibition of BRCA1 phosphorylation by Dinaciclib restored the sensitivity to cisplatin and doxorubicin in tamoxifen-resistant cells. In addition, we identified that activated PI3K/AKT/ERK pathway in tamoxifen-resistant cells was responsible for the upregulation of BARD1 and BRCA1. BKM120, a PI3K inhibitor, decreased the expression of BARD1 and BRCA1 in tamoxifen-resistant cells and re-sensitized them to cisplatin and doxorubicin both in vitro and in xenografted mice. More importantly, higher BARD1 expression was significantly associated with poorer prognosis in breast cancer patients. BARD1 and BRCA1 were also significantly upregulated in recurrent breast cancer samples after tamoxifen compared with their primary counterparts. These results indicate an important role of BARD1 and BRCA1 in the chemoresistance of ER-positive breast cancer. Targeting PI3K/Akt pathway or BRCA1 phosphorylation may help to overcome the resistance to DNA-damaging chemotherapy in these tumors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-12

Title: CDK4/6 inhibitor resistant ER-positive cells remain dependent on estrogen signalling and retain sensitivity to endocrine therapy

Lenihan C, Bouchekioua-Bouzaghrou K, Abdulghani R, Chupin J, Shia A and Schmid P. Barts Cancer Institute, London, United Kingdom.

Body: Background: Dysregulation of the cyclin D-CDK4/6-Rb pathway is a frequent feature of ER positive breast cancers and has been linked with endocrine resistance. Several randomised trials have shown that CDK4/6 inhibitors in combination with endocrine treatment can substantially improve the outcome of patients with metastatic ER positive breast cancer. With increased clinical use of CDK4/6 inhibitors acquired resistance is emerging as a major clinical challenge. It is critical to understand the sensitivity and responsiveness of these resistant cells to estrogen to consider continued use of endocrine therapy in combination with CDK4/6 inhibition.

Results: Long-term culture of the ER positive cell lines T47D and MCF7 with increasing doses of drug generated distinct clones with acquired resistance to either palbociclib or ribociclib. ESR1 expression was stable across all resistant clones. All clones continued to be reliant on estrogen for cell growth, with estrogen deprivation inducing cell death. Resistant clones still responded to estrogen stimulation. Estradiol treatment induced at least 2-fold transcriptional upregulation of pS2 and c-myc; the induction was inhibited by tamoxifen and fulvestrant treatment. Both palbociclib and ribociclib resistant clones remained sensitive to endocrine therapy, with tamoxifen IC50 values equivalent to the parental T47D and MCF7 cell lines.

Mechanisms of resistance were different between the different CDK4/6 inhibitors and within the two cell lines. Palbociclib resistant MCF7 and T47D clones all increase cyclin E protein levels, but only T47D transcriptionally upregulated cyclin E. MCF7 clones transcriptionally upregulated cyclin D, with corresponding protein increase. Therefore, CDK2/cyclin E complexes seem to drive progression in MCF7 cells, whilst CDK2/cyclin D complexes function in T47D cells. Ribociclib resistant clones presented with unchanged or even decreased CDK2 expression, but demonstrated notable increase in E2F1, indicating the requirement of a non-canonical CDK-cyclin pairing independent of CDK2.

Cell lines did not exhibit complete cross resistance to all CDK4/6 inhibitors. All ribociclib-resistant T47D and MCF7 clones conferred cross-resistance to palbociclib. The majority of clones were also resistant to abemaciclib, although one T47D clone retained abemaciclib sensitivity. All palbociclib-resistant clones were equally resistant to ribociclib. Conversely, whilst one T47D ribociclib clone exhibited marked cross resistance to abemaciclib, the majority of clones remained sensitive to treatment with abemaciclib. This highlights potential different resistant mechanisms for abemaciclib independent characterised cell cycle changes for palbociclib and ribociclib.

Conclusions: Cells resistant to CDK4/6 inhibitors remain dependent on estrogen signalling and retain sensitivity to endocrine therapy. We continue to show that resistance is mediated by non-canonical CDK-cyclin pairings. Palbociclib and ribociclib resistant clones confer cross-resistance to both Palbociclib and ribociclib but incomplete cross-resistance to abemaciclib.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-13

Title: Chronic inhibition of signal transducer and activator of transcription 3/5 in treatment-resistant human breast cancer cell subtypes: Convergence on the reactive oxygen species/SUMOylation pathway and its effects on xCT expression and system x_c^- activity

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Body: Pharmacologically targeting activated signal transducer and activator of transcription 3 (STAT3) and/or STAT5 has been an active area of cancer research. The cystine/glutamate antiporter system x_c^- contributes to redox balance and export of intracellularly produced glutamate in response to up-regulated glutaminolysis in aggressive breast cancer cells. We have previously shown that blocking STAT3/5 using the novel small molecule inhibitor SH-4-54, designed to target the SH2 domains of both proteins, increases expression of xCT, which encodes the active component of system x_c^- , thereby increasing antiporter activity in human breast cancer cells. The current investigation demonstrates that chronic treatment with SH-4-54, followed by clonal selection of treatment-resistant MDA-MB-231 and T47D breast cancer cells, elicits distinct subtype-dependent effects. xCT mRNA and protein levels, glutamate release, and cystine uptake are decreased relative to untreated passage-matched controls in the triple-negative MDA-MB-231 SH-4-54-resistant clones, with the inverse occurring in estrogen-responsive T47D cells. This “ying-yang” effect is linked with a shifted balance between phosphorylated STAT3 and STAT5, intracellular levels of reactive oxygen species (ROS), STAT5 SUMOylation/de-SUMOylation, and the expression of STAT3/5 target genes (assessed by NextGeneration RNA sequencing). STAT5 emerged as a definitive negative transcriptional regulator of xCT, while STAT3 activation was coupled with increased system x_c^- activity. Specifically, inhibiting constitutive STAT3 phosphorylation in MDA-MB-231 cells induces ROS, thereby affecting the SUMO pathway by favoring de-SUMOylation and STAT5 phosphorylation. Activated STAT5 is then able to serve as a repressor by binding to its recognition sequence within the xCT promoter, reducing xCT expression and thereby destabilizing an important redox balancing mechanism by limiting cystine uptake through system x_c^- . In contrast, in ER α -positive cells that initially respond to STAT5-mediated signaling, STAT3 becomes activated and xCT expression is up-regulated in response to chronic SH-4-54 treatment, potentially leading to a more aggressive cancer subtype. Further destabilizing the cellular redox status may therefore be critical to produce clinically meaningful outcomes linked to chronic treatment with potent STAT3/5 inhibitors like SH-4-54. We assessed this notion by treating SH-4-54-resistant MDA-MB-231 clones with specific ROS-inducing reagents, including capsazepine, bleomycin, and paclitaxel, which produced different effects on cell viability. We propose that careful classification of a patient's breast cancer subtype is central to therapeutically targeting STAT3/5 as a means of treating breast cancer, particularly given that xCT is emerging as an important biomarker of aggressive cancers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-14

Title: ER α propelled drug-resistance-facilitating global DNA hypermethylation by promoting the DNMT1 gene expression

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Body: Drug-induced aberrant DNA methylation is the first identified epigenetic marker involved in chemotherapy resistance. Tumor cells exposed to toxic concentrations of commonly used cancer chemotherapy agents usually develop global DNA hypermethylation, both in vitro and in vivo. Understanding how the aberrant DNA methylation is acquired would impact cancer treatment in theory and practice. In this study we systematically investigated whether and how ER α propelled aberrant global DNA hypermethylation in the context of breast cancer drug resistance. Our data demonstrated that anticancer drug paclitaxel (PTX) augmented ER α binding to the DNMT1 and DNMT3b promoters to activate DNMT1 and DNMT3b genes, enhancing the PTX resistance of breast cancer cells. In support of these observations, estrogen enhanced multi-drug resistance of breast cancer cells by up-regulation of DNMT1 and DNMT3b genes. Nevertheless, the aberrant global DNA hypermethylation was dominantly induced by ER α -activated-DNMT1, since DNMT1 over-expression significantly increased global DNA methylation and DNMT1 knockdown reversed the ER α -induced global DNA methylation. Altering DNMT3b expression had no detectable effect on global DNA methylation. Consistently, the expression level of DNMT1 was positively correlated with ER α in 78 breast cancer tissue samples shown by our immunohistochemistry (IHC) analysis and negatively correlated with relapse-free survival (RFS) and distance metastasis-free survival (DMFS) of ER α -positive breast cancer patients. This study provides a new perspective for understanding the mechanism underlying drug-resistance-facilitating aberrant DNA methylation in breast cancer and other estrogen dependent tumors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-15

Title: Synergistic suppression of triple negative breast cancer with the combination of PI3K inhibitor (alpelisib, BYL719) and CDK inhibitor (ribociclib, LEE011)

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Body: INTRODUCTION. Triple negative breast cancer (TNBC) is an aggressive form of BC that lacks effective targeted therapy. It is a biologically heterogeneous disease with several molecular subtypes: basal-like 1 (BL1), basal-like 2 (BL2), mesenchymal (M), mesenchymal-stem-like (MSL), immune-modulatory (IM) and unclassified (UNC). The PI3K/AKT/mTOR pathway affects cell proliferation, survival, and apoptosis through growth receptor interaction with downstream targets such as AKT and mTOR. TNBC frequently has activation of this pathway by mutation and other means across several subtypes. Recently the PI3K inhibitor alpelisib (BYL719) has been found to have efficacy in a combination study in ER positive metastatic BC (MBC) with a Phase 3 trial in progress. Ribociclib (LEE011) is a CDK inhibitor targeting cyclin D1/CDK4 and cyclin D3/CDK6 that recently met its primary endpoint in a phase 3 trial in a combination study in hormone receptor positive BC. As with all BC, TNBC has intact or mutant RB which affects susceptibility to CDK inhibitor. We hypothesize that targeting both PI3K with alpelisib and CDK with ribociclib may provide enhanced suppression of TNBC.

MATERIALS AND METHODS. HCC38 and MDA468 are TNBC subtype BL1; MDA468 is RB1 and PTEN mutant. HCC1187 is IM. BT549 is M, mutant RB1 and PTEN. Hs578T, MDA157, and MDA231 are MSL. Cells were treated with alpelisib and ribociclib alone with 2-fold increase in concentrations, or the combination, for 72h and assessed by MTT assay. Western blot was performed using probes for pAkt (T308 and S473), Akt, pS6K1, S6K1, pS6, S6, and β -actin.

RESULTS. Synergy in growth inhibition was seen combining alpelisib and ribociclib as reflected by combination indices <1 (Table). At least 3 subtypes, BL1, M, and MSL have cell lines that have synergy; however, the MSL subtype MDA157 is antagonistic and not RB1 mutant. In the 2 responsive MSL lines Hs578T and MDA231 ribociclib alone has no effect or increases phosphorylation of Akt by Western blot, but in combination with the PI3K inhibitor alpelisib, there is enhanced inhibition of phosphorylation of Akt with enhanced inhibition of the phosphorylation of the end target S6.

Chou-Talalay Combination Indices for Treatment with Alpelisib plus Ribociclib

Cell Line (subtype)	Combination Index Values with Different Combinations of Increasing Effective Proportional Doses of BYL719 and LEE011				
	A	B	C	D	E
RB mutant					
MDA468 (BL1)	>100	1.255	1.194	1.278	0.970
BT549 (M)	0.989	0.994	0.957	0.839	0.726
RB intact					
HCC38 (BL1)	0.232	0.367	0.689	0.680	0.519
HCC1187 (IM)	>100	>100	>100	>100	>100
Hs578T (MSL)	0.204	0.258	0.400	0.494	0.594
MDA231 (MSL)	1.581	0.940	0.721	0.465	0.308
MDA157 (MSL)	7.02	20	51	>100	>100

Conclusion. Our study demonstrates that alpelisib plus ribociclib can synergistically enhance suppression of BC across multiple subtypes of TNBC. Cancer cells within the same subtype may not demonstrate a synergistic response, depending on RB status. Nevertheless, if RB mutant cells are susceptible to ribociclib then synergy can be seen even in these cancer cells. Antagonism

can be seen independent of RB status in the same subtype. These findings point to a potential role for combination therapy with alpelisib and ribociclib in the treatment of TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-16

Title: Molecular iodine impairs chemoresistance and invasive mechanisms, enhances doxorubicin retention and induces downregulation of CD44⁺/CD24⁺ and E-Cad⁺/Vim⁺ subpopulations in MCF-7 cells resistant model

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Body: The main problem in the treatment of breast cancer is the ability of the cells to become chemoresistant and metastatic. Both components involved complex mechanisms based on alterations of apoptosis, the cell cycle and drug metabolism, and its correlates with the cancer stem cell phenotype and/or epithelial-mesenchymal transition. In this study, Doxorubicin (DOX), a member of the anthracycline family used as first-line therapy for several cancers, given chronically to establish a low-dose DOX-resistant mammary cancer model to analyze the effect of molecular iodine (I₂) on the chemoresistant and invasive mechanisms. I₂ exerts an antitumor effect on different types of iodine-capturing neoplasm through its oxidant/antioxidant properties and formation of iodolipids. Wild-type breast carcinoma cells (MCF-7/W) were treated chronically with low DOX concentration (10 nM) to generate a clinically relevant model of DOX-resistance (MCF-7/D). After treatment with I₂ and DOX alone or in combination, the proliferation assay (Trypan Blue exclusion), RT-qPCR expression (p21, Bax, Bcl-2, Survivin, PPAR γ , E-Cadherin and Vimentin), flow cytometry (CD24, CD44, E-Cadherin, and Vimentin) and DOX retention (DOX fluorescence) were performed. Tumorigenic capacity was analyzed by xenograft induction in Foxn1 nu/nu mice. MCF-7/D was established after 30 days of treatment when the culture showed a proliferation rate similar to that of MCF-7/W. These DOX-resistant cells also showed increases in p21, Bcl-2 and MDR-1 expression. Supplementation with 200 μ M I₂ exerted similar effects in both cell lines: it decreased the proliferation rate by approximately 40%, and I₂ co-administration with DOX significantly increased the inhibitory effect (~ 60%) and also increased apoptosis (Bax/Bcl-2 index), principally by inhibiting Bcl-2 expression. The inhibition by I₂+DOX was also accompanied by impaired MDR-1 induction as well as by a significant increase in PPAR γ expression. All these changes could be attributed to enhanced DOX retention and differential down selection of CD44⁺/CD24⁺ and E-Cad⁺/Vim⁺ subpopulations. I₂+DOX selected cells (CD44⁻/CD24⁻ and E-Cad⁺/Vim⁻) showed a weak induction of xenografts, indicating that the iodine supplements reversing the tumorigenic capacity of MCF-7/D. In conclusion, molecular iodine reduces the drug resistance and invasive capacity of mammary cancer cells exposed to DOX and represents an anti-chemoresistance agent with clinical potential. This work was partially supported by grant PAPIIT-UNAM IN201516. We thank Juana Cardenas for technical support. Alexander Bontempo is a student of Programa de Doctorado en Ciencias Biomédicas, UNAM and receives a fellowship from CONACYT 262489.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-17

Title: Can polyploid tumor cells possessing stem cell features be induced in resistant breast carcinomas?

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Body: Cancer stem cells are believed to be responsible for radio- and chemoresistance of malignant tumors. *In vitro* studies demonstrate that ionizing radiation is capable of reprogramming cancer cells from non-stem state into stem state [1, 2]. Moreover, the embryonic stemness cassette was found to be expressed in tumor cells (including breast cancer cells) after they were polyploidized as a result of genotoxic stress [2, 3], thus prompting us to suppose that the polyploid cells and their descendants released by depolyploidization can possess stem cell characteristics. The aim of the current work is to test whether polyploid cells having stem cell features can be also induced *in vivo*, namely in locally advanced breast carcinomas as a result of neoadjuvant chemotherapy (NAC), assuming that this process is not autonomous, but rather stipulated by the tumor microenvironment. The study population consisted of 30 breast cancer patients of age ranged from 31 to 75 y. o. diagnosed in the Latvian Oncology Center of the Riga East University Hospital between 2013 and 2015. The tissue specimens were collected after the patients' informed consent was obtained in accordance with the Ethics regulations. The clinico-pathologic information about these patients, including Ki-67 index and the status of ER, PR and HER2 receptors, was obtained from the aforementioned clinics. The majority of patients (n = 28) had locally advanced breast cancer, predominantly Stage III disease. Both diagnostic biopsy and operation material, such as primary tumors surgically removed after NAC using standard doses of paclitaxel and doxorubicin, were subjected to DNA content analysis with image cytometry. Ploidy-related parameters, such as DNA index and the percentage of cells exceeding 4.5c (presumably proliferating and polyploid cells), were determined. Immunofluorescence staining was applied to evaluate expression of such markers/factors as proliferation (Ki-67), stemness (SOX2 and NANOG) and invasiveness (CD44). At the time of diagnosis, 14 patients had primary tumors possessing near-triploid clones, and these cases in comparison with 16 other cases comprised of near-euploid clones had 4.5-fold increase of percentages of cells exceeding the ploidy of 4.5c ($p < 0.05$) and 1.3-fold increase of percentages of cells positive for Ki-67 ($p > 0.05$). Of 10 cases diagnosed as "triple-negative", 6 were near-triploid. Among those cases that showed the resistance to NAC (grades 1 and 2 by Miller-Payne histopathologic scoring), 67% were near-triploid. Polyploidization, which in some resistant cases is gained by NAC, was likely to be attributed to near-triploid clones. Notably, polyploid cells were positive for Ki-67, SOX2, NANOG, and CD44. Thus, these non-quiescent polyploid cells can possess the invasiveness and self-renewal features that were also seen in descendants after depolyploidization. Perhaps, reversible polyploidy plays a definite role in gaining the resistance of tumor cells to chemo- and radiotherapy *in vivo*.

1. Ghisolfi L. *et al.* PLoS ONE 2012; 7: e43628.
2. Lagadec C. *et al.* Stem Cells 2012; 30: 833-44.
3. Salmina K. *et al.* Exp Cell Res 2010; 316: 2099-112.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-03-18

Title: An ex-vivo platform predicts anti-tumor outcome of metabolically-targeted, algorithm-driven combination therapy in triple-negative breast cancer

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Body: Cancer cells undergo phenotypic cell state transitions in response to chemotherapy as a mechanism that can confer transient resistance. However, such cell state transitions can also unlock unique vulnerabilities that can be exploited using temporally-sequenced combination chemotherapy. Here, utilizing a primary breast cancer ex-vivo functional assay that captures tumor heterogeneity, we report that in response to a chemotherapeutic agent, a subset of cancer cells can mount an acutely-induced phenotypic adaptive resistance to future cytotoxic pressure via the transient acquisition of a unique metabolic state defined by augmented glycolysis together with mitochondrial proficiency. These cells activate two complex, temporally-interdependent pathways that enable a glucose shunt towards the pentose phosphate pathway (PPP), which confers an adaptive cross-tolerance to different chemotherapeutic agents. Mathematically modeling these pathways, and simulating drug schedules, we define a rationally-designed 3-drug combination therapy of metabolic inhibitors and cytotoxic agents, which results in improved cancer survival. Our findings highlight a new bioenergetics-based adaptive resistance mechanism through which cancer cells can survive combinations of chemotherapy. Administration of metabolic inhibitors in rational, temporal sequence with existing chemotherapy can emerge as a new paradigm in the treatment of cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-01

Title: ESR1 mutations, ESR1 fusions and co-occurring alterations assessed in breast cancer tumors

Tan A, Feldman R, Pohlmann P, Lynce F, Swain S, Nunes MR R, Gatalica Z, Ghazalpour A, Swensen J, Vidal G, Obeid E, Vacirca J, Isaacs C and Schwartzberg L. Levine Cancer Institute - Carolinas HealthCare System, Charlotte, NC; Caris Life Sciences, Phoenix, AZ; Lombardi Comprehensive Cancer Center - MedStar Georgetown University Hospital, Washington, DC; University of Tennessee - West Cancer Center, Memphis, TN; Fox Chase Cancer Center, Philadelphia, PA and North Shore Hematology Oncology Associates, East Setauket, NY.

Body: Background: ESR1 mutations and fusions arise in hormone receptor positive (ER+ and/or PR+) breast cancer (HR+ BC) patients after aromatase inhibitor (AI) therapy (low estrogen states), to become constitutively active in a ligand-independent manner. Patients with ESR1 mutations exhibit worse prognosis and outcome with no particular benefit of chemotherapy vs tamoxifen treatment (Augusto, et al. ASCO 2016). A retrospective analysis of the ESR1 mutation frequency and co-occurring alterations that could guide subsequent therapy approaches was investigated.

Methods: Molecular profiles of 416 breast cancer patients [HR+ (n=237), HER2+ (n=29) and TNBC (n=139)] were assessed. Protein expression (IHC) and gene amplification (ISH) were performed. Genomic testing included 592-gene hybrid-capture NGS [NextSeq Illumina platforms] and ArcherDx fusion assay based on anchored multiplex PCR (AMP) FusionPlex Solid Tumor. ESR1 variant (ESR1var) (mutation/fusion) profiles and HR+ BC patients lacking genomic ESR1 alterations (ESR1 WT) were compared; Pearson's chi-squared test was used to test for significant differences.

Results: An ESR1 mutation (point mutations, insertion-deletions [n=49] and fusions [n=4]) was detected in 13% (53 /416) of the specimens, and this constitutes 21% (50/237) of all HR+ breast cancers. Two TNBC patients exhibited ESR1 variants (H398Y in exon 7 and A491S in exon 9, both are classified as variants of unknown significance). ESR1 mutations were not detected in HER2+ BC. Seventy-seven percent of patients with ESR1 mutations were detected in metastatic specimens (p=0.03), with liver (19/53 or 36%) and bone (8/53 or 15%) specimens as the most frequent sources for ESR1 variant (ESR1var) positivity. The most common alleles detected were: D538G (24%), Y537S (18%), E380Q (14%) and L536H (4%); 15 other additional alleles were detected (each 2%). ESR1 fusions were detected in 4 ESR1 WT patients: ESR1-ATP2B2, ESR1-MKL1/ESR1-TNRC6B, ESR1-ARNT2 and ESR1-C6ORRF211. We next compared ESR1var (mutation/fusion) profiles to HR+ breast patients lacking genomic ESR1 alterations (ESR1 WT). ER expression was present in 100% and 96% of ESR1var and ESR1 WT BC, respectively, however expression of PR was negative in 22% and 38% of ESR1var and WT BC, respectively (p=0.05). Significantly higher rates of other gene amplification events observed in ESR1var vs. ESR1 WT BC included: c11orf30 [EMSY, BRCA2 interacting transcriptional repressor] (20% vs. 7%), CCND1 (51% vs.28%), CCND2 (6% vs. 0%), FGF3 (37% vs. 16%), FGF4 (40% vs. 12%) and FGF19 (40% vs. 15%), whereas cMYC was more frequently amplified in ESR1 WT BC (17% vs. 2%); all p-values <0.05. KRAS mutations were higher in ESR1var vs WT BC (4% vs. 0%; p=.004). Alterations in the PIK3CA pathway were common in both ESR1var and ESR1 WT BC: mutations in PIK3CA, AKT and PTEN were observed in 27% and 26%; 4% and 7%; 6% and 5%, respectively, and PTEN loss by IHC in 29% and 26%.

Conclusions: ESR1 mutations and fusions are detected in 21% of HR+ BC, the majority of which are in metastatic sites. Amplifications of genes involved in downstream regulatory pathways were present and may contribute to the poor prognosis of ESR1var HR+ BC. Correlation with antecedent therapy is currently underway.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-02

Title: Differential turnover of estrogen receptor alpha in invasive lobular carcinoma

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Body: Background: Invasive lobular carcinoma (ILC) accounts for 10-15% of invasive breast cancers diagnosed annually. There is increasing evidence that endocrine treatment response might differ between Invasive Ductal Carcinoma (IDC) and ILC, and that patients with ILC have worse long-term survival when other prognostic factors are taken into account. One such factor is ER status, which is more likely to be positive in ILC (90-95%) compared to IDC (60-70%). There are few studies that have directly compared mRNA and protein levels between ER+ ILC and ER+ IDC.

Hypothesis: Differences in ER protein steady state levels, and/or turn-over rates contribute to differences in endocrine treatment response between patients with ILC vs IDC.

Methods: We utilized publicly available TCGA data to compare ER mRNA and protein levels between ER+ ILC (n=184) and IDC (n=534). Correlation analysis with Spearman's rank order coefficient (ρ) was used to study the relationship between mRNA and protein levels. METABRIC data were analyzed to compare ER mRNA levels between ER+ ILC (n=130) and IDC (n=1152). ER H-scores and mRNA levels were also analyzed from patients with ER+ ILC (n=180) and IDC (n=1183) seen at our local UPMC Magee Womens Hospital. Finally, ER mRNA and total protein levels, and RNA and protein turn-over rates were determined in 2 IDC and 2 ILC breast cancer cell lines, using qRT-PCR and immunoblots analysis.

Results: Analysis of ESR1 gene expression in the TCGA database revealed significantly lower levels of ER mRNA (Mann-Whitney, $p < .0005$) in ER+ ILC compared to IDC, whereas ER protein levels were similar in the two histological subtypes. The correlation between ER mRNA and protein levels is weaker in ER+ ILC ($\rho = 0.60$) compared to ER+ IDC ($\rho = 0.69$) tumors, though not statistically significant. The weaker correlation between mRNA and protein expression in ILC is more clear when analyzing all 130 RNA and protein pairs with available RPPA data, (ILC median $\rho = 0.28$; IDC median $\rho = 0.34$, $p < .0005$). In the METABRIC dataset, ESR1 mRNA levels were also found to be lower in ER+ ILC tumor samples compared to IDCs (Mann-Whitney, $p < 0.005$). In concordance with these observations, the study of patients seen at our local hospital showed similar ER IHC H-scores for ER+ ILCs (H-score = 244) and IDCs (H Score = 248), despite there being significantly lower ESR1 mRNA in ILC ($p < 0.005$). Finally, our *in vitro* data showed that rate of estrogen-mediated turn-over of ER protein was significantly lower in the ILC cell lines compared to the IDC cell lines, which might explain the lack of lower ER protein levels despite lower ER mRNA levels. We are currently confirming these findings in additional cell lines, and deciphering the mechanisms through the study of ER ubiquitin-modification and proteasome machinery comparing ILC and IDC.

Conclusion: We have provided functional and *in silico* data that collectively suggest altered ER protein turn-over in ILC compared to IDC. We are currently testing if and how this affects sensitivity of ILC cells to SERDs, and underlying mechanisms.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-03

Title: S100 β as a predictive biomarker and monitoring tool in endocrine resistant breast cancer

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Body: In estrogen receptor positive breast cancer, endocrine therapy is the standard line of treatment and even though it results in reduced recurrence and mortality, a significant number of patients will eventually relapse. Early detection of metastatic disease would significantly enhance management of endocrine resistant breast cancer. Here we investigate the potential of the calcium-binding protein S100 β as a predictive biomarker and monitoring tool in endocrine treated patients. Furthermore, the efficacy of S100 β inhibition as therapy in patients that fail first line endocrine therapy was examined.

Primary tumor tissue expression of S100 β protein was assessed in a retrospective cohort of endocrine treated breast cancer patients. Expression of S100 β indicated a significant reduction in time to disease recurrence (n=509, Wilcoxon p<0.0001, hazard ratio 2.43, 95% C.I. is 1.607 to 3.69, p<0.0001, Cox proportional hazard model).

S100 β protein is also detectable in serum of breast cancer patients and elevated levels of serum S100 β prior to removal of primary tumor is associated with poor disease free survival in endocrine treated patients (n=190, Wilcoxon p=0.0367, hazard ratio 2.68, 95% C.I. is 1.12 to 6.41, p=0.026, Cox proportional hazard model). Serum levels of S100 β are significantly reduced after primary tumor resection (n=19, p=0.0003). In serial samples taken during the treatment period, elevated levels of S100 β significantly associated with disease progression and with the emergence of metastatic disease (p=0.0031).

In an *in-vivo* model of endocrine resistant breast cancer, raised levels of S100 β marked the emergence of disease progression. The oncogene steroid receptor co-activator 1 (SRC1) and its interaction with homeobox protein (HOXC11) regulates S100 β production in a src-kinase dependent manner. Here, src-kinase inhibition reduced tumor burden with a concomitant reduction in serum S100 β . We also observed a marked reduction in expression of proliferative marker Ki67 and S100 β protein following the treatment of endocrine resistant patient tumor explants with src-kinase inhibitor.

Associations between elevated levels of serum S100 β and subsequent disease progression in endocrine treated patients, suggests S100 β as a monitoring tool for early detection of disease progression. Additionally high level of S100 β can be used as a potential companion diagnostic tool for stratifying patients on endocrine therapy suitable for treatment with small molecule src-kinase inhibitor.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-04

Title: Detection of *ESR1* mutations in matched primary and metastatic samples from endocrine-resistant lobular breast cancer patients

Desmedt C, Pingitore J, Rothé F, Rouas G, Bertucci F, Galant C, Rotmensz N, van den Eynden G, Salgado R, Larsimont D, Pruneri G and Sotiriou C. Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; Institut Paoli-Calmettes, Marseille; Cliniques Universitaires Saint Luc, Brussels, Belgium and European Institute of Oncology, University of Milan, Milan.

Body: Background:

Invasive lobular breast cancer (ILBC) represents the second most common histology of breast cancer (BC) and accounts for 10-15% of all invasive cases. Since >90% of ILBCs express the estrogen receptor (ER, coded by the *ESR1* gene), the vast majority of these patients receive endocrine therapy. *ESR1* mutations have mainly been identified in metastases from ER-positive BC at a frequency ranging from 11 to 50% and were shown to be associated with resistance to endocrine therapy. Nevertheless, *ESR1* mutations have never been assessed in metastatic ILBC, hence the present study.

Patients and methods:

We aimed at interrogating the five most commonly reported *ESR1* mutations (Y537S/C/N, D538G, E380Q) by droplet digital PCR (BioRad) in matched primary, axillary and metastatic ILBC samples (N=212) from 69 endocrine-resistant patients collected retrospectively from five hospitals.

Results:

We present here the results for the two most frequent *ESR1* mutations (Y537S and D538G); data from the remaining mutations will be available at the time of the conference. We observed Y537S and D538G mutations in metastases from three and four patients, respectively. For one patient, the sampled metastasis harbored both the Y537S and the D538G mutations, confirming that *ESR1* polyclonality can be present in the same metastasis. For another patient, two metastases were sampled and the D538G mutation was only present in one metastasis. Intriguingly, for two patients we observed D538G mutations only in the primary tumor but not in the corresponding metastasis, and for another only in an axillary lymph node. We could hypothesize that the clone carrying the mutation in the early setting has been removed either by the primary surgery or subsequent adjuvant chemotherapy. The *ESR1* Y537S and D538G mutational frequencies observed in our metastatic ILC cohort (3/69, 4.35% and 4/69, 5.80%, respectively) are not statistically different from the frequencies reported in breast cancer metastases in the literature across the different studies (6.62% and 6.20%, respectively). All patients with *ESR1*-mutated metastases received at least 4 years of endocrine therapy and all but one were treated with an aromatase inhibitor (AI). However, half of these patients received exclusively endocrine therapy in the adjuvant setting.

Conclusion:

This is to the best of our knowledge, the first metastatic ILBC series in which the most frequently reported *ESR1* mutations are being investigated, and the largest series in which *ESR1* mutations are being investigated in matched metastatic, primary tumor and axillary lymph node samples. The frequencies that we found for the Y537S and D538G mutations are in line with those reported in the literature in metastatic biopsies for the general BC population. We further demonstrated using multiple samples from the primary tumor and an ultra-sensitive technology that there was no patient presenting an *ESR1* mutation both in the early and metastatic disease. Data on the remaining mutations (Y537S/N, E380Q) will complete these results.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-05

Title: Identification of SAR439859, an orally bioavailable selective estrogen receptor degrader (SERD) that has strong antitumor activity in wild-type and mutant ER+ breast cancer models

Shomali M, Cheng J, Koundinya M, Weinstein M, Malkova N, Sun F, Hebert A, Cindachao M, Hoffman D, McManus J, Levit M, Pollard J, Vincent S, Besret L, Adrian F, Winter C, El-Ahmad Y, Halley F, Hsu K, Lager J, Garcia-Echeverria C and Bouaboula M. Sanofi, Cambridge, MA and Sanofi, Vitry-Sur-Seine, France.

Body: Estrogen receptor positive (ER+) breast cancer accounts for 70% of all breast cancers and is primarily treated with endocrine therapy. Approximately 40% of patients on endocrine therapy will become resistant via a number of mechanisms. There is evidence that in many cases ER continues to play a central role, including mutations in ER leading to a constitutively active receptor. Estrogen receptor degraders like fulvestrant are effective in shutting down ER signaling; however, poor pharmaceutical properties limit fulvestrant clinical activity and prevent it from achieving maximum receptor blockade. We describe the discovery of SAR439859, a novel, orally bioavailable SERD that is a potent antagonist and degrader of ER both in vitro and in vivo. SAR439859 has robust activity in multiple ER+ breast cancer cell lines including cells that are resistant to tamoxifen as well as cell lines harboring ER mutants. Across a large panel of ER+ cells, SAR439859 demonstrated broad and superior ER degradation activity than most SERDs undergoing clinical testing. This leads to a profound inhibition of ER signaling, better inhibition of cell growth and results in improved in vivo efficacy. SAR439859 demonstrated tumor regression in all ER+ BC models including MCF7-ESR1 mutant-Y537S model, as well as patient-derived xenograft model that is resistant to endocrine therapies. Furthermore, SAR439859 displays limited cross-resistance with other class of SERDs. Taken together, these results suggest that SAR439859 would be of therapeutic benefit in metastatic BC setting for patients harboring wild type or mutant ER. SAR439859 is being advanced toward the clinic.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-06

Title: ER reactivation rapidly elicits cell death effects in anti-estrogen-resistant breast cancer

Hosford SR R, Kettenbach AN N, Varn FS S, Cheng C and Miller TW W. Dartmouth College, Lebanon, NH.

Body: Antagonism of estrogen receptor (ER) transcriptional activity using adjuvant anti-estrogen therapies has improved disease outcomes in many patients with ER+ breast cancer. However, cancer recurs in 1/3 of patients within 15 years of follow-up. While anti-estrogens can slow the progression of metastatic disease, this disease is almost uniformly fatal. Prior to the development of tamoxifen, high-dose estrogens were used to treat late stage breast cancer with response rates similar to those achieved with tamoxifen. Increased efficacy of estrogen therapy was observed in women who were farther past menopause, suggesting that tumor adaptation to low-estrogen conditions is associated with response to estrogen therapy. Similarly, withdrawal of anti-estrogen therapy in patients with anti-estrogen-resistant disease has shown clinical benefit.

MCF-7 cells with acquired resistance to fulvestrant (fulv; FR), and long-term estrogen-deprived (LTED) MCF-7 and HCC-1428 cells overexpress ER compared to parental controls. Upon withdrawal of fulv in FR cells or treatment with 17 β -estradiol in LTED cells, ER transcriptional activity is re-engaged at higher levels than in parental cells, concomitant with drastically decreased cell proliferation and increased apoptosis in endocrine-resistant lines. ER reactivation coincides with an unfolded protein response (UPR) following fulv withdrawal (FR) or E2 treatment (LTED). However, treatment of LTED cells with a proteasome inhibitor protects against apoptosis induced by E2 treatment. Prior studies in other cancer subtypes have shown that proteasome inhibitor treatment can prevent expression of pro-apoptotic FasL, which is upregulated following ER reactivation in FR and LTED cells. Alternatively, inhibition of the proteasome may prevent degradation of anti-apoptotic Bcl-2 family proteins including Mcl-1, which is downregulated following FW in FR cells.

The WHIM16 PDX model was derived from a post-menopausal patient with anti-estrogen-resistant ER+/PR+ breast cancer that responded to 17 β -estradiol therapy. WHIM16 PDX tumors grown in ovariectomized mice rapidly, completely regress upon 17 β -estradiol treatment. Tumor regression is paralleled by increased Src activation, which is associated with ER turnover and has been implicated in 17 β -estradiol-induced apoptosis. Src activation is also observed in FR cells following fulv withdrawal, and in LTED cells treated with E2. Treatment of LTED cells with the Src inhibitor dasatinib protects against E2-induced apoptosis, indicating Src activity may be required for the anti-cancer effects of 17 β -estradiol.

Upon withdrawal of 17 β -estradiol, clinically silent (non-palpable) WHIM16 tumors resume growth; however, tumors remain sensitive to repeat administration of 17 β -estradiol. Long-term fulv-withdrawn FR cells show restored sensitivity to fulv, indicating that cycling of estrogen and anti-estrogen therapies may be an effective treatment strategy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-07

Title: The new oral SERD AZD9496 is efficacious in antagonizing ER and circumventing resistance to endocrine therapy

Nardone A, Weir H, De Angelis C, Cataldo ML, Fu X, Shea MJ J, Mitchell T, Trivedi M, Chamness GC C, Osborne CK Kent and Schiff R. Lester & Sue Smith Breast Center, Houston, TX; Dan L. Duncan Comprehensive Cancer Center, Houston, TX; Baylor College of Medicine, Houston, TX; AstraZeneca, Oncology iMED, Alderley Park, Macclesfield, United Kingdom and University of Houston College of Pharmacy, Houston, TX.

Body: Background: The selective estrogen receptor (ER) degrader (SERD) fulvestrant (Ful) is a potent ER antagonist that upon binding to ER induces its degradation. Ful has shown clinical efficacy in metastatic disease upon progression on previous endocrine therapies and superior activity compared to an aromatase inhibitor as first line therapy when given at a high dose, 500mg. However, major clinical limitations of Ful are its low bioavailability and its route of administration. Here, we assess the efficacy and the mechanism of action of the new oral SERD AZD9496 compared to Ful in our panel of endocrine-sensitive and -resistant (EndoR) *in vitro* and *in vivo* models.

Methods: The effects of AZD9496 and Ful were studied *in vitro* in various ER+ MCF7, ZR75-1, T47D, 600MPE, and MDAMB415 parental lines and in MCF7 and T47D derivatives made resistant (R) to estrogen deprivation (ED), tamoxifen (Tam), or Ful. Cell growth, Western blot, Q-RT-PCR, and ERE-reporter assays were conducted to assess treatment efficacy as well as ER levels and activity. Xenografts of parental MCF7 cells were established in ovariectomized nude mice with exogenous estrogen (E2). Mice were then randomized to continued E2 or ED, with and without AZD9496 or Ful. Mice bearing transplantable MCF7 EDR and TamR xenografts were randomized to continue original treatment or to switch to Ful or AZD9496, and tumor size was followed. Expression of classic and nonclassic/indirect ER-regulated genes was evaluated in RNA extracts of short-term-treated xenografts using the BioMark FLUIDIGM platform.

Results: AZD9496 inhibited cell growth (50-100%) of all ER+ parental cells and greatly, though not fully, degraded ER protein levels. AZD9496 also potently reduced ER-dependent exogenous and endogenous gene/protein expression in presence and absence of E2. In parental MCF7 xenograft-bearing mice, 10 days of AZD9496 resulted in a greater inhibition of tumor growth and in a greater reduction of levels of ER-dependent targets in comparison to Ful in the presence of E2. The effects of the 2 SERDs were similar in the absence of E2. In EndoR models that retain ER, AZD9496 inhibited cell growth *in vitro* by degrading ER, similar to Ful. Both SERDs also delayed tumor growth of EDR and TamR xenografts and effectively reduced levels of ER and ER-induced proteins, though no tumor regression was observed in the TamR model. Notably, AZD9496 failed to inhibit growth of FulR cells and xenografts. Expression analysis showed that the 2 SERDs potently inhibited classic ER activity, while simultaneously increasing expression of some genes known to be regulated by the nonclassic/indirect ER activity, including genes involved in escape pathways of endocrine resistance.

Conclusions: The oral SERD AZD9496 is a potent antiestrogen that antagonizes and degrades ER. AZD9496, like Ful, inhibits ER-dependent transcription and tumor growth in both naïve and resistant EDR and TamR models, but shows cross-resistance in FulR models. Both AZD9496 and Ful failed to completely reduce ER protein expression and to induce TamR tumor regression, suggesting that additional strategies to reduce ER levels and to enhance the inhibition of ER signaling and/or of co-operating survival mechanisms may be needed to improve treatment outcome.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-08

Title: The role of HER2 mutations in resistance to endocrine therapy in ER+ breast cancer

Nayar U, Cohen O, Oh C and Wagle N. Dana-Farber Cancer Institute, Boston, MA and Broad Institute of MIT and Harvard, Cambridge, MA.

Body: Resistance to endocrine therapies in estrogen receptor positive (ER+) metastatic breast cancer is widespread, and understanding the mechanisms whereby these tumors acquire resistance is a critical need. Through whole-exome sequencing of metastatic tumor biopsies from patients with endocrine resistant ER+ metastatic breast cancer, we identified 13 different HER2 mutations, including five in the kinase domain, four in the signaling domain, three in the extracellular domain, and one in the transmembrane region of the protein. Two of the kinase domain mutations (L755S and V777L) have been previously described and shown to be activating and resistant to reversible anti-HER2 targeted therapies; the remaining mutations have not been reported. In several of these patients, whole exome sequencing of a pre-treatment primary tumor did not identify the HER2 mutations seen in the corresponding metastatic tumor, suggesting that they were acquired during therapy. To examine the role of HER2 mutations in endocrine resistance, we generated ER+ breast cancer cell lines (MCF7 and T47D) stably expressing the HER2 mutants observed in our clinical data. Several mutants promoted enhanced growth in charcoal dextran-stripped media, which lacks estradiol and mimics treatment with aromatase inhibitor. In addition, several mutants conferred varying degrees of resistance to fulvestrant and tamoxifen. Taken together, these results suggest that HER2 mutations are associated with acquired resistance to endocrine therapies in patients with ER+ breast cancer. The ability of irreversible anti-HER2 agents as well as other agents that target the HER2 pathway to overcome this resistance is being tested for individual HER2 mutations *in vitro*. The results from these studies may provide a clinical rationale for therapeutic combination strategies in patients with refractory tumors that have acquired endocrine resistance through HER2 mutations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-09

Title: Genomic analysis to evaluate response to neoadjuvant anastrozole and fulvestrant in post-menopausal ER-positive HER2-negative breast cancer patients included in the UCBG CARMINA02 trial

Callens C, Bessoltane N, Ngo C, Chemlali W, Becette V, Bernard V, Delattre O, Lemonnier J, Mouret-Reynier M-A, Andre F, Bieche I and Lerebours F. Institut Curie, Paris, France; Unicancer, Paris, France; Institut Gustave Roussy, Villejuif, France and Centre Jean Perrin, Clermont Ferrand, France.

Body: Background: CARMINA02 is a non-comparative multicenter phase II randomized trial evaluating the clinical response rate after up to 6 months of neoadjuvant endocrine therapy (NET) in HR+/HER2- patients with 1 mg anastrozole (Arm A) or 500 mg fulvestrant (Arm B). Secondary objectives included predictive markers of response and outcome. Between 2007 and 2011, 116 women with operable infiltrating breast adenocarcinoma T2-T4 N0-N3 M0 were randomized. Clinical response rates at 6 months (RECIST criteria) were 52.6% [95%CI 41-64%] in Arm A and 36.8% [95%CI 25-49%] in Arm B (Cancer 2016, in press). We aimed to identify the molecular predictive markers of resistance or sensitivity common to both treatments.

Methods: Ninety tumor RNA from clinical responder (n=34) and resistant patients (n=23) treated in arms A or B have been sequenced with Illumina HiSeq2500 technology leading to 2x100-nt paired-end RNA-seq reads. These samples are from pre-treatment (29 in arm A, 28 in arm B) and post-treatment tumors (6 months after, 17 in arm A and 16 in arm B). Alignment was performed with Tophat_2.0.6. Differential gene expression was analyzed with the Differential Expression analysis for Sequence count data package. Gene fusion was detected with ChimeraScan, TophatFusion and DeFuse tools. Variant calling including variations, insertions and deletions was processed following GATK recommendations for RNAseq datas. Quantitative RT-PCR experiments were done to confirm RNA-seq expression results in patient samples not selected for RNA-seq analysis (validation cohort).

Results: We first analyzed differentially expressed genes (DEGs) between responders and non-responders in pre-treatment or post-treatment samples to select potential predictive markers of response. We identified 51 DEGs before treatment common to anastrozole and fulvestrant. Among these 51 genes, *SGK2* was the only gene more intensely expressed in responders than in non-responders. Then we compared DEGs between pre and post-treatment samples for responders or non-reponders for both treatment arms. *SGK2* expression remains stable after treatment. Furthermore we identified 7 DEGs specific to responders and 11 DEGs specific to non-responders. Concerning genes fusion detection predicted by at least 2 tools, none was specific to a response type. We noted a higher number of fusions in non-responders samples. Variants detected by RNA-Seq are being confirmed by DNA-Seq using a home-made next-generation sequencing panel including 95 genes frequently mutated in breast cancers (analysis ongoing).

Conclusion: High expression of *SGK2*, encoding a kinase induced in response to signals that activate PI3kinase, may represent a predictive marker of sensitivity to NET. DEGs associated with NET response or resistance belong to cell cycle, DNA replication and repair, cell death and drug metabolism. Ongoing DNA-seq datas will complete this genomic analysis. This research was conducted with support from AstraZeneca and Institut Curie.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-10

Title: Utility of the orally bioavailable selective estrogen receptor degrader AZD9496 in ESR1 mutant preclinical models of estrogen receptor positive breast cancer

Morrow CJ J, Lawson M, Ladd B, de Almeida C, James N, Curwen JO O, Hanson L, Bell K, Goeppert A, Fisher D, Gangl E, De Savi C, Scott JS S, D'Cruz C, Klinowska T and Weir HM M. Oncology iMed, Astrazeneca, Cambridge, United Kingdom; Oncology iMed, Astrazeneca, Waltham, MA; Oncology iMed, Astrazeneca, Macclesfield, United Kingdom and Discovery Science, Astrazeneca, Cambridge, United Kingdom.

Body: Approximately 70% of breast cancers express estrogen receptor alpha (ER α) and anti-hormonal therapies which either block the production of estrogen (e.g. anastrozole) or directly block ER α function (e.g. tamoxifen) remain the mainstay of treatment for these patients. While these therapies are highly effective resistance can occur. A common resistance mechanism to anti-hormonal agents is the mutation of ESR1, the gene that encodes ER α , which leads to ligand independent activity. Evidence is emerging that the selective estrogen receptor degrader (SERD) fulvestrant is effective in patients with ESR1 mutations. However, given the low bioavailability of fulvestrant and the still detectable levels of ER in clinical samples after treatment with fulvestrant it is hypothesised that SERDs with improved pharmacokinetic properties which are able to drive greater degradation of ER α may provide additional clinical benefit. We have previously described the discovery and characterisation of the orally bioavailable SERD AZD9496, which is currently in phase I clinical trials in ER+ breast cancer patients. Here we report the preclinical activity of AZD9496 in cell line and patient derived xenograft models expressing clinically relevant ESR1 mutations. We have engineered MCF-7 cells to express Y537S ESR1 which confers the ability to proliferate in the absence of estradiol, consistent with ligand independent activation of ER signalling. AZD9496 is able to inhibit proliferation of these cells and downregulate progesterone receptor protein expression at low nanomolar concentrations. Furthermore, when MCF-7 Y537S ESR1 cells are implanted as xenografts they grow in the absence of exogenous estradiol, are as sensitive to AZD9496 as parental MCF7 xenografts and demonstrate downregulation of ER dependent biomarkers. AZD9496 also has anti-tumour and pharmacodynamic efficacy in patient derived xenograft models expressing D538G ESR1. Taken together, these data strengthen the body of data suggesting that SERDs may be active in patients with tumours containing ESR1 mutations and supports the inclusion of this patient population in AZD9496 clinical trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-11

Title: ER is required for mTORC1 inhibitor-induced feedback activation of PI3K/AKT in ER+ breast cancer cells and patients' tumors

Yang W, Chen VS S, Schwartz GN N, Marotti JD D, Rosenkranz KM M, Gui J and Miller TW W. Norris Cotton Cancer Center, Geisel School of Medicine at Dartmouth, Lebanon, NH.

Body: The mTORC1 inhibitor everolimus (afinitor) is approved for the treatment of patients with advanced/metastatic ER+/HER2- breast cancer in combination with the steroidal aromatase inhibitor exemestane following progression on a non-steroidal aromatase inhibitor. The BOLERO-2 and TAMRAD studies demonstrated that combined everolimus/anti-estrogen therapy provided longer PFS compared to anti-estrogen alone. However, it has not been clarified whether continued treatment with an anti-estrogen backbone is beneficial in the setting of mTORC1 inhibition.

Upon activation by mTORC1, p70S6K phosphorylates the insulin-like growth factor-1 receptor (IGF-1R)/insulin receptor (InsR) effector IRS-1 to promote IRS-1 degradation, which in turn decreases activation of phosphatidylinositol 3-kinase (PI3K), AKT, and mTORC1. *IGF1R*, *IRS1*, and *IRS2* are ER-inducible genes, and crosstalk between the ER and IGF-1R pathways has been described. We hypothesized that mTORC1 inhibition with everolimus will upregulate IGF-1R/InsR/IRS-1/2 signaling to activate PI3K/AKT and promote cancer cell survival, while combined inhibition of ER and mTORC1 will block PI3K/AKT activation by decreasing IGF-1R and IRS-1/2, providing rationale for combined targeting of ER and mTORC1.

In 3 ER+ breast cancer cell lines, everolimus treatment increased phospho-AKT levels. ER inhibition with fulvestrant suppressed the induction of P-AKT by everolimus. IGF-1R/InsR inhibition with OSI-906, and RNAi-mediated knockdown of IGF-1R, InsR, or IRS-1/2, decreased everolimus-induced P-AKT. Everolimus sensitized IGF-1R/InsR to IGF-1 that was suppressed by fulvestrant but enhanced by 17 β -estradiol. Although fulvestrant decreased IGF-1R and InsR protein levels, phospho-receptor tyrosine kinase profiling showed that fulvestrant increased P-IGF-1R and P-InsR. Acting downstream of IGF-1R/InsR, fulvestrant prevented everolimus-induced PI3K/AKT activation by blocking binding between the p85 regulatory subunit of PI3K and IRS-1, possibly by decreasing IRS-1/2 levels. In summary, everolimus-induced activation of PI3K/AKT requires IGF-1R/InsR/IRS-1/2 signaling facilitated by ER. Combined treatment with fulvestrant and everolimus synergistically inhibited growth in 4 ER+ cell lines.

To determine whether ER promotes PI3K/AKT activation induced by mTORC1 inhibition in patients' tumors without exposing patients to everolimus, we analyzed live tumor tissues from post-menopausal patients with ER+/HER2- breast cancer treated +/- letrozole for 10-21 d before surgical tumor resection. Tumor cores (1 mm diameter) were used for *ex vivo* culture in DMEM +/- everolimus +/- OSI-906 for 1 h, and lysates were analyzed by immunoblot. Everolimus significantly increased P-AKT in tumors from untreated patients (n=10). OSI-906 did not affect P-AKT, but OSI-906 suppressed everolimus-induced P-AKT. In tumors from letrozole-treated patients (n=7), neither everolimus nor OSI-906 affected P-AKT. These data collectively suggest that ER activation is required for activation of PI3K/AKT induced by mTORC1 inhibition, and provide rationale for therapeutic combinations of anti-estrogens and mTORC1 inhibitors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-12

Title: Both spliced and unspliced XBP1 regulates breast cancer cell fate response to antiestrogen via NFκB signaling

Clarke R, Hu R, Warri A, Jin L, Zwart A, Riggins R and Fang H-b. Georgetown University, Washington, DC.

Body: Unfolded protein response (UPR), a stress-induced survival mechanism, may be used by cancer cells to avoid cell death. Antiestrogen therapy, widely applied in the treatment of estrogen receptor-positive (ER+) breast cancer, induces endoplasmic reticulum stress (ER stress) that leads to activation of each of the three arms of the UPR. One critical prosurvival activator that is regulated by two arms of the UPR is the transcription factor X-box binding protein 1 (XBP1). XBP1 exists in two isoforms, the transcriptionally inactive unspliced XBP1(U) and the spliced, active XBP1(S). Overexpression of XBP1(S) confers estrogen independence and antiestrogen resistance in ER+ breast cancer cells and XBP1(S) expression correlates with poor clinical responsiveness to Tamoxifen in ER+ breast tumors. However, the underlying signaling mechanisms regulated by XBP1, which may mediate its effects on antiestrogen resistance, are unknown. We show that depletion of endogenous XBP-1 by siRNA increases apoptosis, decreases autophagy, requires down-regulation of p65/RelA, a component of the pro-survival NFκB complex. Using novel spliced and non-spliceable forms of XBP1, we show that XBP1(U) and XBP1(S) both regulate NFκB activity via ERα signaling in breast cancer cells. XBP1(S), but not XBP1(U), also can regulate p65/RelA expression independent of ERα. Antiestrogen resistance as conferred by XBP1 overexpression in MCF-7 cells requires the activation of NFκB signaling; inhibition of NFκB signaling by either the small molecule NFκB inhibitor Parthenolide or p65/RelA siRNA sensitizes XBP1 overexpressing cells to Tamoxifen. The activation of XBP1 and the downstream NFκB signaling is likely to contribute to the TMEM33 overexpression induced apoptosis, as both NFκB signaling and XBP1(S) are elevated in TMEM33 overexpressed MCF7 cells. Thus, we have identified a critical regulatory link between the UPR/XBP1 pathway and pro-survival NFκB signaling that is a major contributor to endocrine responsiveness in ER positive breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-13

Title: Protein tyrosine kinase 6 (PTK6) promotes survival of endocrine therapy-resistant ER+ breast cancer cells

Irie HY Y, Park SH H, Katsyv I, Zhang W and Nayak A. Icahn School of Medicine at Mount Sinai, New York, NY.

Body: Background/Rationale: The non-receptor tyrosine kinase PTK6/Brk is highly expressed in the ER+/Luminal breast cancer subtypes. PTK6 expression has prognostic significance for patients with ER+ disease; higher transcript levels are associated with poorer survival. The functions of PTK6 in the context of ER+ breast cancer and sensitivity to endocrine therapy have not been explored. We sought to determine the functional roles of PTK6 in ER+ breast cancer cells, including those that are relatively resistant to current endocrine therapies. **Methods/Results.** We modulated the expression of PTK6 using both gain- and loss-of-function approaches. Enhanced expression of PTK6 in Tamoxifen-sensitive ER+ breast cancer cells was sufficient to confer relative Tamoxifen resistance. Furthermore, downregulation of PTK6 in ER+ breast cancer cells, including those that have acquired resistance to Tamoxifen, induced apoptosis, as evidenced by an increase in AnnexinV+ cells and increased levels of cleaved PARP. PTK6 downregulation impaired growth of Tamoxifen-resistant variants of ER+ MCF7 and T47D cells (MCF7TamR and T47DTamR) in 3D Matrigel culture, and virtually abrogated primary tumor growth of MCF7TamR xenografts. Mechanistically, p38MAPK activation is critical for PTK6 downregulation-induced apoptosis of ER+ breast cancer cells, as p38 inhibition partially rescues cells from PTK6 shRNA-associated apoptosis. **Conclusions:** Our studies highlight the critical role that PTK6 plays in the survival of ER+ breast cancer cells, including those that are resistant to endocrine therapy. Enhanced PTK6 expression in ER+ breast cancer cells is sufficient to promote endocrine therapy resistance, which could contribute to the poorer prognosis associated with higher PTK6 expression in ER+ patient tumors. As small molecule PTK6 inhibitors are becoming available, our studies support further evaluation of PTK6 as a candidate therapeutic target for endocrine therapy resistant ER+ breast cancers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-14

Title: Zoledronic acid sensitized breast cancer cells to fulvestrant via ERK/FOXO3a/HIF-1 α inhibition through nuclear FOXO3a translocation

Jia X, Liu G, Shen Z and Shao Z. Key Laboratory of Breast Cancer in Shanghai, Fudan University Shanghai Cancer Center, Shanghai Medical College, Fudan University, Shanghai, China.

Body: Background: Previous studies have shown that HIF-1 α conferred endocrine resistance and zoledronic acid decreased HIF-1 α expression in estrogen receptor positive breast cancer. We investigated the combination effect of zoledronic acid and fulvestrant and the possible mechanism for the inhibition of HIF-1 α by zoledronic acid.

Materials and methods: We established a mouse xenograft model stably expressing HIF-1 α received PBS, fulvestrant, zoledronic acid and fulvestrant plus zoledronic acid during the whole experimental period. The tumor volume was compared and the expression of HIF-1 α and FOXO3a was detected by immunohistochemistry and western blot. Cell proliferation, clonogenic survival, western blot and immunofluorescence were determined by fulvestrant and zoledronic acid. All statistical tests were two-sided.

Results: The combination of zoledronic acid and fulvestrant was shown to be synergistic in the mouse xenograft model. As such, zoledronic acid demonstrated synergy in combination with fulvestrant in vitro (Figure 1A). Zoledronic acid did not inhibit the tumor growth of MCF-7 cells stably expressed HIF-1 α . Furthermore, zoledronic acid inhibited ERK1/2 phosphorylation, while the PI3K/AKT pathway was not significantly affected (Figure 1B). The combination of zoledronic acid and AZD6244, a MEK/ERK pathway inhibitor restored the inhibition of HIF-1 α by zoledronic acid. However, the combination of zoledronic acid and BKM120, a PI3K inhibitor did not restore the inhibition of HIF-1 α by zoledronic acid. Importantly, zoledronic acid increased FOXO3a phosphorylation and nuclear FOXO3a translocation with no changes in total FOXO3a expression in vitro (Figure 1C). In addition, the inhibition of HIF-1 α by zoledronic acid was partial restored in human breast cancer T47D (Figure 1D) and MCF-7 cells (Figure 1E) transiently transfected with FOXO3a-specific short-interfering RNA (siRNA).

Conclusion: This study showed that zoledronic acid significantly increased the sensitivity of breast cancer cells to fulvestrant through inhibition of ERK/FOXO3a/HIF-1 α pathway.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-15

Title: The PI3K-inhibitor, copanlisib, has selective activity in luminal breast cancer cell lines and shows robust combined activity with hormonal blockade and CDK-4/6 inhibition in ER+ breast cancer cell line xenografts

O'Brien NA A, Conklin D, Luo T, Ayala R, Issakhanian S, Kalous O, Von Euw E, Politz O, Wilhelm S, Childs BH H, Hurvitz SA A and Slamon DJ J. UCLA, Los Angeles, CA and Bayer Pharmaceuticals, Berlin, Germany.

Body: Background: Genetic and epigenetic alterations in the PI3K/mTOR and cyclin D:CDK-4/6:Rb signaling axes occur frequently in breast cancer and have been attributed to resistance to both ER- and HER2-directed therapeutics. Pharmacologically targeting CDK-4/6 in combination with hormonal blockade provides clinical benefit in patients with advanced ER+ breast cancer. In this study, we evaluated the activity of the pan-class I PI3K inhibitor, copanlisib (BAY-80-6946), with potent alpha and delta activity as a single agent or in combination with CDK-4/6 inhibition and hormonal blockade in a panel of breast cancer cell lines.

Methods: The growth inhibitory activity of copanlisib was evaluated against a large panel of 48 breast cancer cell lines molecularly characterized by genomic, transcriptomic and proteomic profiling. IC₅₀ values were determined from direct cell counts using a Z1-particle counter. The activity of copanlisib in combination with hormone blockade and CDK-4/6 inhibition, by palbociclib, was assessed in two cell line xenograft models of ER+ breast cancer; MCF7(*PIK3CA-E545K*) and ZR751(*PIK3CA WT*). For xenograft studies, tumor bearing mice were treated once weekly (BID) by intravenous injection with clinically achievable doses of copanlisib (10 mg/kg) as single agent or in combination with tamoxifen or fulvestrant with or without 75 mg/kg daily palbociclib for 21 days.

Results: A broad range of IC₅₀ values (0.491-895 nM), with a high degree of separation between sensitive and resistant histologically defined subgroups were determined for copanlisib, indicating the potential for a wide therapeutic window. Luminal subtype, the presence of activating mutations in *PIK3CA*, high levels of ER, HER2, HER3 and EGFR protein enriched for sensitivity to copanlisib. Activating mutations of KRAS and BRAF were associated with resistance to copanlisib. Single agent copanlisib induced significant tumor growth inhibition (TGI) relative to vehicle control in each of the xenograft models. Modest increases in anti-tumor activity were achieved when copanlisib was combined with hormonal blockade by either tamoxifen or fulvestrant. However, robust tumor regressions were observed with the triple combinations of copanlisib-palbociclib-tamoxifen and copanlisib-palbociclib-fulvestrant. Furthermore, these triple combinations achieved a statistically significant improvement in anti-tumor activity over the standard of care combination of palbociclib plus fulvestrant. Each of the single agent and treatment combinations tested were well tolerated in animals.

Discussion: These preclinical data illustrate the potent and selective activity of the pan class I PI3K inhibitor copanlisib in luminal breast cancers and support the clinical investigation of copanlisib in combination with CDK-4/6 inhibition and hormonal blockade in ER+ breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-16

Title: Steroid receptor co-activator 1 mediation of cancer cell reprogramming in endocrine resistant breast cancer

Browne A, Fagan A, Vareslija D, Ward E, Hill A and Young L. Royal College of Surgeons in Ireland, Dublin, Ireland and RCSI Beaumont Hospital, Dublin, Ireland.

Body: Cancer cells undergo dynamic and frequently reversible modifications and this cellular plasticity permits reprogramming in response to long term endocrine treatment. A well-established theory, the cancer stem cell theory; suggests that within an ER positive tumour cell population are a small number of stem cells which are capable of infinite self-renewal and are insensitive to treatment with standard endocrine regimes. However, emerging reports that pluripotent cells can be generated from adult somatic cells (Takahashi, 2007) alludes to an alternate mechanism for cancer cells to reprogramme and evade endocrine treatment. To investigate the role of tamoxifen in promoting cellular reprogramming and endocrine resistance in breast cancer, we isolated single luminal A breast cancer CD24⁺CD44⁻ clones from endocrine sensitive MCF-7 cells. The clones were expanded in the presence and absence of tamoxifen. Extensive profiling of cells following long-term exposure to tamoxifen revealed a resistant phenotype similar to that reported in other established models.

Steroid receptor co-activator 1 (SRC-1) is a master regulatory protein which has been shown to be central to the development of endocrine resistance. The mechanisms of which are still poorly understood (Walsh *et al.*, 2012). Utilising genome-wide transcriptomic sequencing this study investigated whether SRC-1 can regulate transcriptional networks which mediate reprogramming in individual cells to induce survival adaptability and drug resistance in breast cancer.

RNA-sequencing was carried out in a model of endocrine resistance (shNon-Targeting versus shSRC-1). There were 1,731 genes up-regulated by SRC-1, of which 153 were identified as transcription factors (TFs)/chromatin remodellers. Combining the transcriptomic profiling with CHIP-sequencing we identified a transcriptional network pertinent to SRC-1. Molecular characterisation identified E2F7, NFIA, DEK, SMAD2, SMARCA1, ASCL1 and TRPS1 as key drivers of SRC-1 mediated endocrine resistance. Each TF was confirmed as a direct target of SRC-1, via promoter specific binding and were found to be drivers of endocrine resistant cell migration. Furthermore, all TFs were necessary in mammosphere formation and their promotion of cellular de-differentiation was observed by 3D acinar organisation and flow cytometry. To elucidate the core TFs required for cancer cell reprogramming, their effect on the pioneer reprogramming TFs (OCT4, SOX2, cMYC and KLF4) was investigated. SMAD2, SMARCA1, ASCL1 and TRPS1 have emerged as pivotal regulators of endocrine resistant cell reprogramming.

This study has unravelled an SRC-1-mediated TF network responsible for promoting the cellular reprogramming of breast cancer cells. Concerted activity of this network is responsible for driving de-differentiation of cells and enhancing their stem-like, highly migratory and proliferative tumour initiation population. This study provides important information regarding the mechanism of cellular reprogramming in ER positive endocrine resistant cancer and may lead to potential novel therapeutic targets.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-17

Title: Global transcriptional repression by the coactivator SRC-1 mediates disease progression in treatment-resistant breast cancer

Ward E, Varešlija D, Fagan A, Hill A and Young L. Royal College of Surgeons, Dublin, Ireland and Beaumont Hospital.

Body: Despite the effectiveness of endocrine therapy in treating estrogen receptor (ER) positive breast cancer, nearly 40% of breast cancer patients may develop resistance which can lead to metastatic disease progression. Steroid receptor co-activator-1 (SRC-1), a key regulator of ER signalling, is overexpressed in 35% of breast cancer patients and is strongly associated with the development of endocrine resistant metastasis⁽¹⁾. Recent studies highlighted the ability of SRC-1 to also act as transcriptional repressor. SRC-1 can therefore bi-directionally regulate gene expression to promote the resistant phenotype⁽²⁾. This study employed a genome-wide multi-omics sequencing approach to determine the SRC-1 repression signature in endocrine resistance and elucidate the mechanism by which SRC-1 mediates this repression. RNA-sequencing identified 736 genes significantly downregulated by SRC-1, with common functional pathways such as differentiation, cell morphogenesis and extracellular matrix enriched in the gene set. Parallel global methylation sequencing analysis revealed distinct differentially methylated regions specific to SRC-1 repressed target genes. Mechanistic studies in endocrine resistant cells revealed a role for methylation proteins, DNMTs and MBD, in SRC-1 directed repression. Through combined analysis of our global sequencing data we identified a network hub of five differentiation genes directly repressed by SRC-1. High expression of this signature predicts enhanced recurrence free survival in tamoxifen treated patients ($n=335$, $p=0.032$). A reduction in expression of these genes was shown to have functional output on proliferation, migration and mammosphere formation. Finally, we use tumour explant models to show that targeting DNA methylation can be used to reverse the SRC-1 driven suppression of this differentiation hub. Here we report a novel mechanism by which SRC-1 may be driving endocrine resistant tumorigenesis. Our genome-wide discovery approach revealed a global epigenetic re-programming pathway whereby concerted differential DNA methylation is potentiated by the activation of SRC-1 in the presence of tamoxifen in the endocrine resistant setting. This study suggests that therapeutic strategies of combined targeted epigenetic therapy with estrogen deprivation could be a successful strategy to prevent acquired resistance to endocrine therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-18

Title: The impact of circulating androgens on the androgen receptor in aromatase inhibitor resistant breast cancer

Creevey L, Ali A, Bleach R, Hill ADK DK, Young L and McIlroy M. Royal College of Surgeons in Ireland, Dublin, Ireland.

Body: Aromatase Inhibitor (AI) therapy is the gold standard first line therapy for post-menopausal breast cancer. Following AI treatment the conversion of circulating androgens into estrone can be diminished by >99%, completely altering the tumour steroid microenvironment. Estrogens have been extensively studied in terms of the role of sex steroids as causative agents of breast cancer but interestingly, a case-control study nested within the European Prospective Investigation into Cancer and Nutrition reported that elevated levels of both serum androgens and estrogens are associated with increased breast cancer risk (E2 RR: 2.28 and androstenedione RR: 1.94). Also clinical studies have reported increases in the serum levels of androstenedione (4AD) in patients that recur on AI therapy.

In order to begin to address the role of circulating androgens and the androgen receptor (AR) in AI resistant breast cancer, RNA sequencing was performed on AI resistant (MCF7-aro-LetR) cells cultured with 4AD in the presence of letrozole. Gene expression analysis was performed using the cBioPortal software found that PI3K signalling pathways were upregulated in response to 4AD treatment as well as the upregulation of pathways involved in exocytosis and hormone secretion. We found elevated levels of PI3K and AR in endocrine resistant breast cancer compared to sensitive cell lines and that our AI resistant LetR cells showed significant reduction in cell growth in response to the combined pan class PI3K inhibitor BEZ235 and anti-AR treatment. Clinically, we evaluated the potential role of PI3K signalling in AI resistance a breast cancer patient TMA (n=488) was stained immunohistochemically for phospho-AKT and AR. The protective effect conferred by high AR expression in the total patient population (p=0.02) and tamoxifen treated population (p=0.05) was lost in the AI treated population (p=0.49). For p-AKT staining, patients that were positive for p-AKT significantly associate with the more aggressive luminal B classification yet exhibit an inverse association with luminal A subtype. Kaplan Meier analysis revealed that AR is associated with a more favourable outcome in p-AKT negative patients but that this is lost in the context of elevated p-AKT. Furthermore, interrogation of the breast invasive carcinoma TCGA dataset (n=963) revealed that patients with upregulated AR mRNA and wildtype PI3K exhibited more invasive disease compared with those harbouring a PI3K mutation (p=0.02) (n=56). Further investigations into the AR interacting partners will help elucidate potential mechanisms of action and the role of androgens and AR in facilitating breast cancer tumourigenesis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-19

Title: Inhibition of ER-positive breast cancer progression with duavee, a new form of hormone replacement therapy

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Body: Menopause occurs in all women between the ages of 45 and 55 and often results in undesirable vasomotor symptoms. Hormone replacement therapy (HRT) can alleviate these symptoms, including hot flashes, difficulty sleeping, fatigue, and vaginal atrophy, and also prevents osteoporosis. PremPro, a HRT formulation that combines conjugated equine estrogens (CE) with medroxyprogesterone acetate, was found to increase the risk of breast cancer in the Women's Health Initiative (WHI) trial. Due to the perceived risk based largely on the results of the WHI trial, the number of women taking HRT has dramatically decreased. Studies suggest that breast cancer cases from PremPro treatment were primarily due to the outgrowth of occult tumors, not the formation of new disease. Duavee, a new form of HRT that combines CE and bazedoxifene (BZA), a selective estrogen receptor modulator (SERM) and degrader (SERD), has been approved by the FDA for treatment of moderate to severe hot flashes and to reduce the risk of osteoporosis. Importantly, this CE+BZA mixture not only relieves symptoms associated with menopause, but it also does not stimulate the breast or uterus. Several preclinical studies suggest that CE+BZA might be protective in the breast, however the mechanism of action of this new combination therapy is not known. Our goal, therefore, is to elucidate the underlying molecular mechanisms by which CE+BZA differentially affects estrogen receptor alpha (ER α) action in the mammary gland, using transcriptome and whole genome occupancy analysis in breast cancer cell lines. We are also studying the effects of CE+BZA on early mammary cancer progression in the polyoma middle T antigen (PyMT) transgenic mouse model, which is sensitive to estrogens. In addition, we are studying the effects of CE+BZA in an ER α -positive patient-derived xenograft (PDX) mouse model. We have determined that CE modulates gene expression in MCF7 cells similar to 17 β -estradiol (E2), and that BZA is able to inhibit these effects. We have also observed that CE increases ER α occupancy, similar to E2, at response elements associated with some estrogen target genes, whereas CE+BZA decreases this occupancy. In the PyMT mouse model, CE+BZA delays the onset of mammary tumors in ovariectomized mice and prolongs their survival when compared to E2 and CE treatment alone. In the PDX model, the CE+BZA tumor growth curve is below vehicle, although it does not quite reach statistical significance. An improved understanding of the molecular mechanisms of CE+BZA action in hormone sensitive breast cancer cell and animal models should have important implications for women considering HRT.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-20

Title: Predicting therapeutic effect by on tumor invasion lymphocytes (TILs) in endocrine therapy for stage IV breast cancer

Kashiwagi S, Asano Y, Goto W, Takada K, Takashima T, Morisaki T, Noda S, Onoda N, Ohsawa M, Hirakawa K and Ohira M. Osaka City University Graduate School of Medicine, Osaka, Japan and Department of Diagnostic Pathology, Osaka City University Graduate School of Medicine, Osaka, Japan.

Body: Background: Breast cancer with distant metastasis at first presentation (stage IV disease) is often encountered in the outpatient department. With recent advances in multimodal therapies for breast cancer, long-term survival can now be expected even in stage IV breast cancer with distant metastasis. However, a goal in treating metastatic disease is prolongation of survival while maintaining good quality of life (QOL). Endocrine therapy is suitable for this purpose. The tumor immune environment not only modulates the effects of immunotherapy, but also the effects of other anticancer drugs and treatment outcomes. These immune responses can be evaluated with tumor-infiltrating lymphocytes (TILs), which has frequently been verified clinically. In the present study, we hypothesized that TILs would be useful as predictive marker of the therapeutic effect in endocrine therapy as well. In this study, the prediction of the therapeutic effect by TILs in endocrine therapy for stage IV breast cancer was clinically verified.

Materials and Methods: Data from 40 patients who underwent endocrine therapy as the initial drug therapy for stage IV breast cancer were used. The correlation between TILs evaluated according to the standard method, and prognosis, including the efficacy of endocrine therapy, was investigated retrospectively. Patients with $\geq 50\%$ lymphocytic infiltration were considered to have lymphocyte-predominant breast cancer (LPBC).

Results: Among all 40 patients, TIL levels were high in 13 (32.5%) and low in 27 (67.5%) patients. Nine patients (22.5%) had LPBC, and 31 patients (77.5%) had non-LPBC. Investigation of the clinical pathological features of patients showed no significant differences between the high TIL and low TIL groups. There were also no significant differences between LPBC and non-LPBC patients. An analysis of outcomes comparing the high TIL and low TIL groups showed no prolongation in progression-free survival (PFS) ($p=0.171$, log-rank), time to treatment failure (TTF) ($p=0.054$), or overall survival (OS) ($p=0.641$). LPBC patients had significant prolongations of PFS ($p=0.005$), TTF ($p=0.001$), and OS ($p=0.027$) compared to non-LPBC patients. On receiver operating characteristic (ROC) curve analyses, results were better with LPBC (AUC: 0.700) than with TILs (AUC: 0.606).

Conclusion: The present findings suggest that a high level of lymphocytic infiltration in the tumor stroma may serve as a predictor of the therapeutic effect of endocrine therapy for patients with stage IV ER-positive breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-21

Title: Androgen receptor in tamoxifen-resistant breast cancer is affected by SUMO

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Body: Resistance to tamoxifen is a major problem in treating women with estrogen receptor-positive (ERa⁺) breast cancer (BCa). Previous studies report elevated levels of the androgen receptor (AR) in tamoxifen-resistant (TamR) human mammary tumors. Inversely, *in vitro* overexpression of AR in ERa⁺-MCF-7 cells potentiates growth in the presence of tamoxifen. AR is a target for small ubiquitin like modifiers (SUMO) posttranslational modification (PTM). Prostate cancer (PCa) studies, show that SUMO-PTMs can have diverse effects on AR transcriptional activity, by modifying the AR itself or its co-regulators. SUMOylation regulates the AR's interaction with chromatin and modulates its function in a gene selective manner. Although SUMO-1 inhibited the AR transcriptional activity, SUMO-3 strongly enhanced the transactivation of AR in LNCaP cells but negatively affected the AR transcriptional activity in primary prostate epithelial cells. On the other hand, SUMO modifications and its subsequent effects on the AR in breast tumors and in particular TamR-BCa is unknown. Our objective is to understand how SUMO-PTMs affect AR activity in TamR-BCa. Others report that high Ubc9, a SUMO E2 conjugating enzyme, correlates with with aggressive phenotypes of BCa and that PIAS3, a SUMO E3 ligase, expression increases in breast tumors. Consistently, we now report that global SUMO2/3-conjugation is enhanced while the full-length SUMO protease SENP7 (SENP7L) is reduced in TamR-BCa cells. Collectively, these observations would suggest reduced SUMO dynamics in TamR-BCa as compared to tamoxifen-sensitive (TamS) BCa cells. Unlike PCa cells that favor unmodified over SUMOylated AR, we report that AR SUMOylation is potentiated in TamR-BCa cells. Functionally, SUMOylation of AR augments its chromatin binding and enhances its transcriptional activity. Our results suggest that the level of unmodified to SUMOylated AR is dramatically altered with onset of TamR and drives a functionally distinct population of AR in TamR versus TamS BCa. Consistently these biochemical reactions that define protein diversity could have a major impact on drug resistance in BCa.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-22

Title: RAD1901 demonstrates anti-tumor activity in multiple models of ER-positive breast cancer treatment resistance

Bihani T, Arlt H, Brechbuhl H, Patel H, Tao N, Purandare DM M, Hattersley G, Kabos P and Garner F. Radius Health, Waltham, MA and University of Colorado, Aurora, CO.

Body: Estrogen receptor positive (ER+) breast cancer makes up approximately 70% of all newly diagnosed breast cancers and ER remains the single most useful predictive and prognostic marker for these patients. In fact, dependence on ER signaling is retained in recurrent disease and even in disease treated with multiple lines of anti-endocrine agents. Indeed, inhibiting estrogen synthesis (e.g. with aromatase inhibitors) and modulating ER pathway activity (e.g. with tamoxifen) continue to be mainstays in the standard of care for ER+ breast cancer patients both in adjuvant and metastatic setting. While patients initially respond well to these agents, a majority of patients with metastatic disease will relapse. Therefore, it is critical to improve our understanding and model the specific mechanisms that can lead to treatment resistance. Mutations in the ligand binding domain of ESR1 have been described as a mechanism that contributes to clinical resistance to aromatase inhibitors. In addition, some ESR1 mutations can lead to a conformational change of the receptor, leading to decreased binding of tamoxifen, thereby reducing its activity. Recently, selective estrogen receptor degraders, or SERDs, have gained widespread attention as a novel treatment strategy for ER+ disease. Indeed, fulvestrant has been shown to degrade ER and cause tumor growth inhibition in many ER+ breast cancer models. However, in the clinic, fulvestrant appears to be limited by PK exposure properties and this, combined with its intramuscular route of administration underscore the need for an oral SERD with improved PK properties. Recently, we have described RAD1901, a novel, orally available, non-steroidal small molecule SERD that is able to degrade ER and effectively cause tumor growth inhibition in multiple patient-derived xenograft models (PDX) models, including two that harbor a Y537S mutation in ESR1. To further validate that RAD1901 has the potential to be used in the advanced disease setting, we tested its anti-tumor activity in preclinical models representing the most common ESR1 mutations (D538G, E380Q, S463P, Y537N/C). In addition, we have tested RAD1901 in multiple cell line models of anti-endocrine resistance. These include long-term estrogen deprived cells that model progression of disease on aromatase inhibitors, as well as tamoxifen-resistant models. Here, we present anti-tumor activity of RAD1901 both as a single agent and as part of rational clinical combinations (eg. CDK4/6, PI3K/Akt/mTOR inhibitors) in multiple models of anti-endocrine resistance. These results combined with previous studies demonstrating RAD1901 efficacy in treatment naïve models provide strong preclinical evidence for clinical testing of RAD1901 in both adjuvant and metastatic setting. RAD1901 is currently under clinical investigation for the treatment of ER+ breast cancers in post-menopausal women (NCT02650817, NCT02338349).

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-23

Title: Combination therapy of targeted anticancer pathways and estrogen receptor ligands and their responses in *de novo* and tamoxifen resistant cell models

Gutgesell LM M, Xiong R, Thatcher GRJ RJ and Tonetti DA A. University of Illinois at Chicago, Chicago, IL.

Body: Approximately 75% of breast cancers are classified as Estrogen Receptor positive (ER+). Tamoxifen, a selective estrogen receptor modulator (SERM), is the standard of care for many of these ER+ breast cancer patients. Unfortunately, tamoxifen resistance occurs in almost 50% of patients within 5 years of treatment, and endocrine-independence accompanying resistance also negates the effects of aromatase inhibitors. Combination therapy is increasingly used in non-cytotoxic therapeutic approaches in many types of cancer. The potential exists that endocrine resistance can be lessened, eliminated, or overcome through targeted therapy in combination with endocrine therapy. Inhibition of kinase signaling (e.g. via CDK4/6 or PI3K) and other pathways (e.g. HSP90) are expected to be effective in combination with endocrine therapy. We have discovered a variety of ER ligands with potential as endocrine therapeutic agents, based upon a single chemical scaffold with a diverse set of pharmacological responses: including SERMs, selective ER downregulators (SERDs), selective estrogen mimics (SEMs), and selective human ER partial agonists (ShERPAs). To predict which agents in combination with these endocrine-targeted ligands would be of potential therapeutic benefit it was necessary to develop 3D spheroidal cultures of ER+ breast cancer cell lines: including endocrine-dependent lines; and cell lines made endocrine-independent either by extended exposure to tamoxifen or extended deprivation of estradiol. In contrast to 2D cultures, drug response in 3D spheroidal cell cultures was predictive of response to treatment in mouse xenograft studies. Growth of endocrine-dependent cell lines was, as expected, inhibited by SERDs; and endocrine-independent, tamoxifen-resistant cell lines were also sensitive to SERD treatment, although one cell line was largely resistant. Growth of all three tamoxifen-resistant cell lines was inhibited by SEMs/ShERPAs. Importantly, regardless of the type of endocrine therapeutic agent studied, concentrations leading to saturation of the target (ER) did not cause cell death. Equally, all endocrine therapies studied benefited from combination treatment with other agents, leading to enhanced cell death.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-24

Title: Identification of preclinical mechanisms driving acquired resistance to selective ERa degraders (SERDs), CDK4/6 inhibitors, or to combinations of both agents

O'Brien T, Xiao Y, Ong C, Daemen A and Friedman L. Genentech, South San Francisco, CA.

Body: Estrogen positive (ER⁺) breast cancer accounts for the majority of all breast cancers and standard practice is treatment with endocrine therapy. Recently a series of CDK4/6 inhibitors have emerged that can be effectively combined with estrogen modulators to treat ER⁺ breast cancer patients. The most advanced of these compounds, palbociclib, is now approved in the US in combination with letrozole or fulvestrant. Even though these combinations are effective, invariably patients will relapse. Our understanding of the mechanisms of acquired resistance to CDK4/6 inhibitors is still relatively nascent, and it is not known whether acquired resistance to combination treatments (e.g. palbociclib with fulvestrant) will be the same as mechanisms for the individual single agents. Additionally, it is not known if tumors that no longer respond to one combination regimen will remain sensitive to other standard of care treatments.

To pre-clinically address these questions we generated cell lines that have acquired resistance to three Selective Estrogen Receptor Degraders (SERDs) and to two CDK4/6 inhibitors, palbociclib and abemaciclib. Additionally, we also generated cells with acquired resistance to the combination of fulvestrant and palbociclib. Characterization of cells resistant to each of these treatments will be presented. Initial data indicate that MCF7 cells with acquired resistance to SERDs have increased PI3K signaling and MEK/ERK signaling and have increased sensitivity to inhibitors of these pathways when treated with single agents. These cells are also more sensitive to palbociclib relative to parental cells. All 11 clones with acquired resistance to abemaciclib lose Retinoblastoma (Rb) expression and 20/24 palbociclib-resistant clones lose Rb expression, indicating the importance of Rb to CDK4/6 inhibitor activity. Interestingly, palbociclib resistant clones have decreased sensitivity to fulvestrant and to PI3K inhibitors, and investigation is under way to determine if that relates to slower doubling times for the clones.

Detailed characterization of resistant clones reveals a surprisingly complex interaction between ERa and CDK4/6 signaling, and further results, including acquired resistance to drug combinations, will be presented. These preclinical studies may inform biomarkers of sensitivity and resistance to endocrine agents and CDK4/6 inhibitors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-25

Title: Role of *H19*, a long non-coding RNA, in development of resistance to endocrine therapy in breast cancer cells

Basak P, Chatterjee S, Bhat V, Jin H, Su A, Murphy LC C and Raouf A. Research Institute of Oncology and Hematology, Winnipeg, MB, Canada and University of Manitoba, Winnipeg, MB, Canada.

Body: Introduction:

Majority of breast cancer tumors are Estrogen receptor positive (ER+) where antiestrogen therapies (endocrine therapies) are the best therapeutic strategy to treat this type of tumors. However, eventually over 30% of patients will develop resistance to endocrine therapies resulting in disease relapse. We recently showed that the long noncoding RNA, *H19*, is an estrogen target gene that plays a significant role in estrogen-induced proliferation of the normal and malignant ER+ cells. We therefore hypothesize that *H19* expression is also important to the proliferation of endocrine therapy resistant cells. In this study, we examined if estrogen-independent *H19* expression is important to the development of endocrine therapy resistance.

Objective:

The overall objective of this project is to use therapy sensitive (MCF-7) and therapy-resistant (LCC9) breast cancer cells as model systems to examine the role of long non-coding RNA *H19* in development and maintenance of resistance to endocrine therapy.

Methodology and Results:

We examined the expression of *H19* in ER+ breast cancer cells (MCF7) that under the selective pressure of fulvestrant (ICI, ER down regulator) acquire resistance to ICI. We observed that while *H19* expression was initially decreased as expected, its expression subsequently increased in the ICI-resistant MCF7 cells. Interestingly, *H19* knockdown in MCF7 cells significantly decrease their proliferation as determined by Flowcytometry and made them more sensitive to ICI. We also examined *H19* expression in the ICI-resistant LCC9 cells and found that ICI treatment increased *H19* expression. Interestingly, *H19* knockdown in the LCC9 cells decreased their proliferation and surprisingly made them sensitive to ICI treatment. Previous observations indicate that NOTCH4 receptor (NR4) may be involved in endocrine therapy resistance. Interestingly we found that in presence of ICI, NR4 expression is increased and that forced activation of NR4 markedly increases *H19* expression in LCC9 cells.

Conclusion:

Altogether these observations suggest that *H19* plays an important role in the development of endocrine therapy resistance and further our understanding of the cellular and molecular mechanisms involved in endocrine therapy resistance. These and similar studies could potentially lead to the development of new therapies to treat therapy resistant tumor cells. Further experiments would reveal if signalling pathways that regulate *H19* expression independent of estrogen are useful therapies against endocrine therapy resistant tumors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-26

Title: Establishment and characterization of ST941/C; an ESR1-mutant ER+ breast cancer cell line and xenograft from a patient with acquired resistance to endocrine therapy

Wick MJ J, Diaz A, Thomas M, Moriarty A, Quinn M, Guerra M, Zhu P, Smith P, Tolcher AW W, Puyang X, Patnaik A, Korpai M, Rasco D and Papadopoulos KP P. START, San Antonio, TX and H3 Biomedicine Inc, Cambridge, MA.

Body: Background: The estrogen receptor ($ER\alpha$) plays an integral role in the progression of luminal type breast cancers and while targeted endocrine therapies provide effective initial treatment many patients develop acquired resistance to treatment even with continued ER receptor expression. Recently studies identified ER mutations as a possible mechanism for acquired resistance and several activating point mutations have been identified including Y537S ($ESR1^{Y537S}$) resulting in hormone independent proliferation in preclinical studies. However, lack of validated ESR1-mutant cell lines has limited detailed mechanistic studies of these mutations in endocrine-resistant ER+ breast cancer. Previously we established and evaluated a patient derived xenograft (PDX) designated ST941 representing $ESR1^{Y537S}$ -mutated ER+ breast cancer (Wick et al, SABCS 2015). To better understand the role of ESR1-mutations in endocrine resistant breast cancer we established an immortalized cell line from ST941 designated ST941/C to use for in vitro mechanistic assays and correlative in vivo studies.

Methods: The ST941/C cell line was generated from harvested low-passage ST941 PDX tissue using published methods. Once established the line was characterized by immunohistochemistry and NGS and its tumorigenicity assessed. Drug sensitivity studies were carried out evaluating relevant endocrine and chemotherapy agents and results compared with in vivo drug studies. Timepoints for cell proliferation assays were Days 4, 7 and 10 following cell plating using standard MTS assay. Endpoints for in vivo studies were a mean group tumor volume of $\sim 1\text{ cm}^3$ or sixty days following treatment initiation.

Results: Subcutaneous cell injection into athymic nude mice produced xenografts which grew in the absence of exogenous hormone. The ST941/C cell line and resulting xenograft retained immunohistologic and NGS characteristics of the parent model including receptor expression and ER mutation. Both the cell line and xenograft were insensitive to most endocrine therapies including tamoxifen, fulvestrant and aromatase inhibitors and reported correlative activity towards docetaxel and other chemotherapies.

Conclusion: We have established and evaluated an ESR1-mutant cell line designated ST941/C which is tumorigenic in nude mice and potentially useful for mechanistic and correlative in vivo studies to better understand acquired resistance in endocrine-treated ER+ breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-04-27

Title: Delineating novel molecular pathways driving endocrine resistance in breast cancer

Bosch A, Staaf J, Akrap N, Kaminska KK K, Borgquist S, Borg Å and Honeth G. Lund University, Lund, Sweden.

Body: Background

Estrogen receptor (ER) is a main driver of tumor progression in ER+ metastatic breast cancer (MBC). The use of endocrine therapy can effectively control the disease in a large proportion of patients. However, the majority of MBC eventually become resistant and progress. To elucidate the mechanisms of acquired resistance to endocrine treatment is key in order to better select therapeutic partners and delay disease progression.

Methods

A panel of ER+ breast cancer cell lines initially sensitive to the selective estrogen receptor degrader (SERD) fulvestrant was exposed to increasing concentrations of this drug over several months to induce resistance. Cell proliferation was determined with the xCELLigence system. Protein expression was measured by phospho-kinase array and western blotting. RNA expression was evaluated by gene expression microarray analysis (Illumina) and validated by RT-qPCR. Cell cycle distribution was analyzed by flow cytometry.

Results

Using an unbiased approach to identify pathways that drive endocrine resistance, both the parental and the resistant cell models were studied. As expected, fulvestrant treatment resulted in G1-cell cycle arrest in the parental cell lines.

In stark contrast, resistant cells bypassed fulvestrant-induced proliferation block despite a lower expression of genes driving mitotic progression compared to untreated parental cells. This gene expression pattern was coupled with a reduction of ER protein level in the resistant cells, which was in line with a significant decrease in the expression of ER-target genes. Our phospho-screen analysis showed a genotype specific down-modulation of p53 and up-regulation of several signaling components of mitogenic pathways in resistant cells compared to parental cells.

Conclusions

Our results suggest that acquired endocrine resistance is driven by multiple cell-specific mechanisms rather than a common molecular underpinning. Strikingly, there is one unique feature in our models, which is a cellular switch towards an ER-independent gene expression program. Ongoing in vitro and in vivo studies, aimed at further characterizing these cellular models, will provide valuable insights into the heterogeneity underlying the response to endocrine treatment observed in MBC patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-05-01

Title: Increased glycolysis directly activates transcriptional coactivator SRC-3 to promote aggressive metastatic breast cancer

Dasgupta S, Zhu B, Nikolai BC C, Putluri N, Foulds CE E, Tsai SY Y, Tsai M-J and O'Malley BW W. Baylor College of Medicine, Houston, TX.

Body: Reprogramming of metabolic pathways is one of the central hallmarks of cancer cell growth and survival to support anabolic and energetic demands. Rapidly proliferating tumor cells utilize increased amounts of glucose for energy generation and macromolecule biosynthesis, yet it is still poorly understood how increased glucose-flux accentuates aggressive metastatic tumor. Understanding the molecular and genetic regulators of these metabolic-programs is critical to discover new drug targets for cancer therapy. Here, we report that the oncogenic transcriptional coregulator steroid receptor coactivator-3 (SRC-3) is a critical sensor of increased tumor glycolysis and re-programs transcriptional responses to support breast cancer growth and metastasis. From an unbiased kinome-wide RNAi library screen, we identified the glycolytic enzyme PFKFB4 as a direct stimulator of SRC-3 activity. PFKFB4 is a bifunctional metabolic enzyme that contains both 'kinase' and 'phosphatase' domain, yet the functional role of these regulatory domains in tumor metabolism is not clear. Glucose stimulation promotes PFKFB4-dependent phosphorylation of SRC-3 (pSRC-3) which then enhances transcriptional coactivation by this coregulator. Mechanistic studies revealed that SRC-3 and PFKFB4 mutually cooperate to maintain high levels of purine biosynthesis by transcriptionally upregulating rate limiting enzymes transketolase (TKT), adenosine monophosphate deaminase-1 (AMPD1) and xanthine dehydrogenase (XDH). Knockdown of PFKFB4 or SRC-3 significantly decreased the expression of these enzymes resulting in reduced proliferation of breast tumor cells, and this functional deficiency could be rescued by exogenous addition of purines. Chromatin immunoprecipitation (ChIP) studies identified increased occupancy of pSRC-3 on target gene promoters, which overlapped with activating transcription factor 4 (ATF4) binding sites in the proximal promoter region. Immunoprecipitation of ATF4 identified increased interaction with pSRC-3 in glycolytic breast tumor cells, whereas knockdown of PFKFB4 or expression of a phospho-deficient SRC-3 mutant significantly reduced the SRC-3/ATF4 association. These findings suggest that upon enhanced glycolysis, PFKFB4 induced activation of SRC-3 by phosphorylation promotes expression of purine biosynthetic genes primarily by coactivating ATF4. Importantly, ablation of either SRC-3 or PFKFB4 suppresses *in vivo* breast tumor growth and metastasis, and these tumorigenic-effects cannot be simulated by a phospho-deficient SRC-3 mutant suggesting the PFKFB4-SRC-3 axis may be therapeutically exploited for treating breast cancer patients.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P3-05-02

Title: Quantitative ER α measurements in TNBC from the I-SPY 2 TRIAL correlate with HER2-EGFR co-activation and heterodimerization

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Body: Background: We have previously described that TNBC patients whose tumors have both HER2 Y1248 phosphorylation (pHER2) "high" and phospho-EGFR Y1173 (pEGFR) "high" have increased response (pCR) to neratinib in the I-SPY2 TRIAL. We hypothesize that the paradoxical finding of a response prediction signature comprised of HER2 activation in a HER2 IHC/FISH-negative population means there must be a ligand-driven biochemical event responsible for the HER2 phosphorylation because HER2 mutations were also not found to be significant. Exploratory analysis of additional cellular signaling events and protein expression levels in pre-treatment, LCM-purified tumor epithelium by reverse phase protein microarray (RPPA) included semi-quantitative measurement of total levels of estrogen receptor alpha (ER α), which has been previously shown to be able to act as a membrane non-genomic signaling molecule through direct interaction with various tyrosine kinases including EGFR and HER2. Since ER α has been previously shown to act as a ligand and co-stimulate (activate) HER2 and EGFR when present at low levels, we investigated whether or not RPPA-measured ER α levels in the TNBC cohort analyzed to date were higher in tumors with both pHER2 "high" and pEGFR "high" levels and thus provide evidence explaining how HER2-EGFR activation is occurring in TNBC.

Methods: Using RPPA analysis, we measured 118 analytes in lysates of LCM tumor epithelium obtained from the pre-treatment biopsy samples of 86 TNBC (Allred=0) patients in the I-SPY2 TRIAL analyzed to date. Cutpoints for pEGFR and pHER2 were determined previously by ROC analysis for pCR correlation in the neratinib treated TNBC population, and used here to dichotomize the pHER2 and pEGFR data in the larger TNBC population. Wilcoxon Rank Sum testing was performed using the continuous variable total ER α data and compared the TNBC that were both pHER2 and pEGFR "high" (N=39) to the rest of the TNBC population (N=47). Total ER α values were then divided into "high" and "low" groups based on the TNBC population median value in order to determine frequency/percentages within each class. Our study is exploratory with no claims for generalizability of the data, and calculations are descriptive (e.g. p-values are measures of distance with no inferential content).

Results: Total ER α values were obtained in 84/86 TNBC tumors analyzed. Total levels of ER α were higher (p< 0.006) in TNBC tumors with pHER2 and pEGFR "high" levels. 68% (26/38) of tumors in the pHER2 and pEGFR "high" group had ER α levels above the population median compared to 35% (16/46) in the rest of the TNBC population.

Conclusion: Our exploratory analysis reveals that ER α levels are significantly higher in TNBC with pHER2 and pEGFR activation and may be behaving as a direct signaling ligand in TNBC and driving HER2-EGFR signaling. This ER α -pHER2/pEGFR association was missed by current ER and HER2 clinical laboratory testing techniques, and if validated in larger independent study sets could suggest that utilization of new protein-based techniques defining ER more quantitatively could be helpful to understand tumor biology and therapeutic response prediction, especially in the context of TNBC that are ostensibly ER negative.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-05-03

Title: A novel progesterone receptor (PR)-RNA polymerase III association represses estrogen-dependent growth in breast tumor patient-derived xenografts

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Body: Background: Progesterone (P) is an important hormone for development and normal function of the breast; however, its role in established breast cancers is less clear. P has been implicated in regulating tumor cell growth, signaling, differentiation state, and stem/progenitor properties in breast cancer cells. Progesterone receptors (PRs) are considered positive prognostic indicators yet potential targets for treatment, although the dilemma of positive or negative targeting persists. P can either inhibit or stimulate breast cancer cell growth in the absence of estrogens (E), and usually blocks E mediated growth. Recent studies using breast cancer cell lines have deciphered a mechanism by which P suppresses E dependent growth through modulation of the estrogen receptor (ER) cistrome through a physical ER/PR association. However, the ER/PR association is weak to undetectable in our ER+PR+ breast cancer patient-derived xenografts (PDX). We therefore hypothesize that PR can regulate E-dependent breast cancer growth independent of direct interference of ER transcription. The purpose of this study was to use unbiased genomics and proteomics of breast cancer PDX to uncover additional mechanisms of P repression of breast cancer growth.

Methods: These studies used two luminal ER+PR+ PDX (UCD4 and UCD65) that contain high levels of ER and PR (>90%), and where P inhibits E-dependent growth. Tumors were grown *in vivo* in female NSG mice under continuous placebo, E (17 β -estradiol), E plus P, or E plus the synthetic progestin medroxyprogesterone acetate (MPA) for 8-10 weeks. RNAseq, chromatin immunoprecipitation sequencing (ChIPseq), and rapid immunoprecipitation followed by mass spectrometry of endogenous proteins (RIME) were performed to analyze differential transcriptional, cistromic, and protein-protein interactions in E compared to E plus P or MPA treated tumors. Co-immunoprecipitation (co-IP) and ChIP were used to verify results.

Results: Both P and MPA potently inhibited E-dependent growth of both tumor lines. Gene expression studies found that both hormones reversed transcription of over one third of the estrogen/ER transcriptome in both tumors. RIME for PR uncovered significant interactions between PR and multiple RNA polymerase III subunits (POLR3). Co-IP using POLR3A and POLR3B confirmed PR associates with the POLR3 holoenzyme. Furthermore, ChIPseq revealed that PR binds to one third of POLR3 regulated tRNA genes. PR also associated with Maf1 in one tumor, a known POLR3 suppressor.

Conclusions: Here we describe a novel association of PR with POLR3 in two luminal breast cancer PDX, an interaction not described in breast cancer cell lines. Our data suggest this is a negative regulatory interaction that may occur through recruiting a POLR3 repressor. These data implicate multifaceted P control of E dependent tumor growth; in addition to antagonizing E regulated genes at the transcription level, P can regulate translation through depletion of amino acid carrying tRNAs, thus slowing protein synthesis. Identifying which tumors utilize this growth-suppressive mechanism may pinpoint appropriate candidates for progestin therapy and/or provide a prognostic tool for predicting tumor progression.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-05-04

Title: Impact of androgen receptor expression in fluoxymesterone-treated, estrogen receptor–positive metastatic breast cancer exposed to contemporary hormonal therapy

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Body: Background: The use of the nonselective androgen fluoxymesterone in patients with metastatic breast cancer (MBC) diminished after the 1960s because of its adverse events and a limited understanding of its biological effects. Although fluoxymesterone has had efficacy against tamoxifen-resistant disease in clinical studies, its role in the era of contemporary hormonal therapy is unclear. Recent studies have shown that the androgen–androgen receptor (AR) complex acts as a suppressor of estrogen receptor (ER)+ breast cancer. We hypothesized that fluoxymesterone is effective against MBC that progresses despite contemporary hormonal therapy and that the drug has more clinical benefit in patients with ER+AR+ disease than in patients with ER+AR- disease. We evaluated the survival outcomes of patients with MBC who received fluoxymesterone after contemporary hormonal therapy failed and evaluated the association between ER/AR status and survival outcomes in these patients.

Methods and Materials: We included 103 patients treated with fluoxymesterone who had already received at least one prior hormonal or cytotoxic treatment for MBC between January 1, 2000, and December 31, 2014, at a single institution. A pathologist reviewed these patients' tumors' ER and AR expression levels by immunohistochemical staining. Progression-free survival (PFS) was defined from the start of fluoxymesterone treatment to the date of disease progression or last follow-up. We used Cox regression analysis to examine univariate and multivariate correlates of PFS.

Results: Patients received a median of 3 (range: 0-10) prior hormonal therapies (aromatase inhibitors, tamoxifen, and/or fulvestrant) before fluoxymesterone. Of the 103 patients, 33 (32%) discontinued fluoxymesterone because of physician decision or adverse events, which included toxicity in 14 patients, and 70 (68%) were eligible for tumor response assessment by Response Evaluation Criteria in Solid Tumors. Of these 70 patients, 2 (3%) had a complete response, 7 (10%) had a partial response, and 21 (30%) had stable disease for at least 6 months, yielding a clinical benefit rate of 43%. The median PFS was 3.9 months (95% confidence interval: 3.2–5.3 months). The multivariate analysis revealed no significant association between PFS and the type or number of prior treatments. Thirty-nine patients (38%) had archived tumor slides available for AR staining. All 39 patients had ER+ disease; 5 had $\leq 1\%$, 5 had $>1\%$ but $<10\%$, 18 had $\geq 10\%$, and 11 had no AR nuclear expression. AR positivity defined by the presence of any AR+ cells, $\geq 1\%$ AR+ cells, or $\geq 10\%$ AR+ cells was not significantly associated with survival outcome.

Conclusions: Fluoxymesterone showed objective tumor response and prolonged control of ER+ MBC refractory to contemporary endocrine therapy. The number and type of prior treatments did not impact the drug's clinical benefit, and AR+ status did not influence the clinical outcome. Fluoxymesterone should be considered for patients whose ER+ MBC progresses despite contemporary hormonal therapy, regardless of their AR status.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-05-05

Title: PTHrP affects breast cancer initiation and progression through Stat5 signaling pathway

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Body: PTHrP affects mammary development and breast cancer. PTHrP is expressed in basal myoepithelial cells until late pregnancy, when it also becomes expressed in alveolar epithelial cells. During lactation, PTHrP is secreted into milk and into the circulation, and regulates systemic calcium and bone metabolism. Secretion of PTHrP by breast cancers stimulates bone resorption, promoting the skeletal metastases and/or development of hypercalcemia. Less is known about the contribution of PTHrP to development and/or progression of primary breast cancers. Recent GWAS reports have identified the PTHrP gene as a breast cancer susceptibility locus. Using data from the Cancer Genome Atlas (TCGA) Project and a Yale tissue microarray (YTMA49) we have found that increased breast tumor PTHrP expression predicts a more aggressive phenotype and increased mortality, signifying the clinical relevance of high tumor PTHrP levels. In an attempt to define how PTHrP affects breast cancer, we assessed the effects of PTHrP overexpression on the development of mammary tumors in mice. We developed a tetracycline-regulated, *MMTV*-driven transgenic model of PTHrP overexpression targeted to mammary epithelial cells (*MMTV-rtTA;tetO-hPTHrP*). Surprisingly, we found that PTHrP overexpression in luminal epithelial cells causes alveolar hyperplasia, secretory differentiation and milk production in virgin mice, associated with a lower number of luminal progenitor cells and basal stem cells upon FACS analysis. Additionally, Signal Transducer and Activator of Transcription 5 (Stat5) is highly activated in the setting of PTHrP-induced alveolar hyperplasia. This activation is functionally relevant as evidenced by higher levels of β -Casein mRNA and protein, the transcription of which is regulated by activated Stat5 (pStat5). This effect of PTHrP was reversible upon withdrawal of doxycycline (Dox). Addition of Dox to primary mammary epithelial cell cultures from these mice recapitulated the findings. Knocking out PTHrP receptor (PTH1R) in these mice did not affect this phenotype, suggesting that PTHrP acts in a receptor-independent manner. Overexpression of PTHrP in *MMTV-PyMT* mice dramatically accelerated formation of mammary tumors reducing both latency of tumor formation and survival of the mice. It caused a higher rate of proliferation and a lower rate of apoptosis *in vivo*. All *MMTV-rtTA;tetO-hPTHrP;MMTV-PyMT* mice developed palpable tumors in all mammary glands within 3 weeks of age, became hypercalcemic and died before 4.5 weeks of age. These mice had higher levels of pStat5 and β Casein. Tumor cells cultured *ex vivo* and cell lines established from these tumors overexpressed PTHrP when stimulated with Dox. PTHrP overexpression, and not exogenous PTHrP, caused higher levels of proliferation, pStat5, and β -Casein. This PTHrP-induced activity of Stat5 was blocked by treatment of these cells with a pharmacological inhibitor of Jak2, a tyrosine kinase upstream of Stat5. Additionally, T47D cells transfected with PTHrP showed higher levels of pStat5, suggesting that PTHrP could activate Stat5 signaling regardless of the cell type. We show that PTHrP overexpression results in Stat5 activation and increased proliferation in breast cancer cells in a cell-autonomous fashion and independent of its receptor.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-05-06

Title: Prognostic value of androgen receptor and FOXA1 co-expression in non-metastatic triple-negative breast cancer

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Body: Background

Androgen receptor (AR) is expressed in 8-53% of triple negative breast cancer (TNBC). Its prognostic value in this subgroup is controverted. FOXA1 is essential for expression of 50% of estrogen receptor (ER)-related genes. Microarray studies identified the subgroup of molecular apocrine or luminal androgen receptor tumors that express AR and luminal genes including FOXA1 but nor ER. Preclinical data suggested that FOXA1 may direct AR to sites normally occupied by ER in luminal tumors, inducing an estrogen-like gene program stimulating proliferation. We have already shown that TNBC with AR/FOXA1 co-expression seem to behave like luminal tumors.

We aimed at evaluating co-expression AR/FOXA1-associated profiles and its prognostic value in a large retrospective series of patients with non-metastatic TNBC with a long follow-up.

Patients and methods

AR and FOXA1 expression were evaluated by immunohistochemistry in tissue microarrays of 300 patients with non-metastatic TNBC treated in our center between 2002 and 2012. Positivity threshold was set at $\geq 10\%$ staining.

Results

Median age was 57.7 years (range 28.5-98). 46.2% of tumors were classified T1 and 64% pN0. We found 83.2% of ductal carcinomas, 5% of lobular carcinomas and 11.8% of other histological types. SBR grade 1-2 represented 21.4%. A basal-like phenotype (cytokeratins 5/6 and/or EGFR+) was observed in 63.7% of cases. In 161 evaluable patients, a *PIKCA* mutation (exon 9 or 20) was observed in 16.2% of cases. In 124 evaluable patients, a deletion of PTEN was observed in 25% of cases. Adjuvant chemotherapy was delivered in 74.5% of patients.

37.7% of patients had AR+ tumors and 29.7% had AR+ and FOXA1+ tumors. AR+/FOXA1+ tumors were more frequently: found in older patients ($p < 0.001$), lobular ($p < 0.001$) and of lower nuclear grade ($p < 0.001$) than others TNBC. AR+/FOXA1+ tumors exhibited less frequently basal-like phenotype (45.8%) than AR+/FOXA1- (77.3%) and AR-/FOXA1- (70.1%) tumors ($p < 0.001$). AR+/FOXA1+ tumors exhibited more frequently *PIK3CA* mutations (35.8%) than AR+/FOXA1- (16.7%) and AR-/FOXA1- (5.9%) tumors ($p < 0.001$). AR+/FOXA1+ tumors exhibited less frequently PTEN deletion (6.8% vs 16.7% for AR+/FOXA1- vs 36.5% for AR-/FOXA1- tumors, respectively, $p = 0.001$).

With a median follow-up of 5.5 years, recurrence-free survival (RFS) was significantly lower for patients with AR+/FOXA1+ tumors ($p = 0.046$). 3-years RFS were 84.2% and 85.3% for AR+/FOXA1+ and the others TNBC, respectively. 5-years RFS were 67.4% and 79.4% for AR+/FOXA1+ and the others TNBC, respectively. Tumor size, nodal status and adjuvant chemotherapy were also statistically correlated to RFS. Tumor size, nodal status, histology and adjuvant chemotherapy were significantly associated with overall survival.

Conclusions

In this large series, almost 30% of TNBC had an AR/FOXA1 co-expression with distinct clinicopathological characteristics and a worse outcome than others TNBC with higher risk of late recurrences. These biomarkers could be useful to identify a subgroup of TNBC and could have therapeutic implications: anti-androgen are under investigation, FOXA1 could be another therapeutic target and PI3K inhibitors should be evaluated in this specific subgroup, alone or in association with anti-androgens.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-05-07

Title: Flutamide reduced tumor progression and altered steroid hormone secretion in human and canine inflammatory breast cancer cell lines

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Body: Background: Inflammatory breast carcinoma (IBC) is a special type of breast cancer with a poor survival rate and accounts for 6% of diagnosed breast cancers. The role of androgens on breast cancer is on rise in research, trying to propose anti-androgen therapeutic strategies. The aim of this study was to determine the effects *in vivo* and *in vitro* of flutamide (anti-androgen drug) on cell proliferation, tumor progression and steroid production in two cancer IBC triple negative cell lines (SUM-149 and IPC-366, human and canine, respectively). **Material and Methods:** IPC-366 was cultured in Dulbecco's modified Eagle medium nutrient mixture F-12 Ham (DMEM/F12) and SUM149 was maintained in Ham's F-12 media. Flutamide concentrations added to the culture media were: 5 μ M, 10 μ M, and 15 μ M for 72 hours. Additionally, IPC-366 and SUM149 xenotransplanted mice were used for *in vivo* assays with the same flutamide concentrations administrated subcutaneously. Steroid hormones determination in culture media and tumor homogenates (pregnenolone (P5), progesterone (P4), dihydroepiandrosterone (DHEA), androstenedione (A4), testosterone (T), dihydrotestosterone (DHT), 17 β -estradiol (E2) and estrone sulphate (SO4E1)) were assayed by EIA previously validated. Immunohistochemical (IHC) analysis of the steroidogenic enzymes CYP11A1, 3 β -HSD, CYP19A1, 17 β -HSD and 5 α -reductase were assayed. **Results:** Percentage of cell proliferation showed a decrease in all treatments in IPC-366 and SUM149. *In vivo* tumor progression was reduced in around 65% in IPC-366 and SUM149 xenotransplanted mice. Regarding hormonal secretion assayed in pellets and homogenates. in treated groups there was an increased in steroid secretion as showed the high levels found in P5, P4 and A4. T and DHT concentrations were higher in treated groups, in contrast to E2 levels that decreased. 17 β -HSD and 5 α -reductase by IHC showed a high expression in treated groups. **Conclusion:** IPC-366 and SUM149 treated with flutamide reduced the proliferation of neoplastic cells, reduced tumor progression in xenotransplanted mice and altered steroid hormone secretion by increasing T production and decreasing in E2 levels. These results open a future approach for IBC and triple negative breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-05-08

Title: Steroid receptors and steroidogenic enzymes in human breast cancer: Associations with breast cancer subtypes and clinical outcome

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Body: Introduction: The majority of breast cancer (BC) cases are believed to be highly steroid dependent. Therefore exploitation of currently untapped steroid pathways could potentially contribute to our search for novel targeted breast cancer therapies. Given the growing understanding of the importance of both intratumoral steroid metabolism and impact of alternate steroid receptors in BC biology, we evaluated selected steroidogenic pathways in a cohort of 140 BC cases from Akershus University Hospital, Norway. In this study we immunolocalized both the androgen receptor (AR) and glucocorticoid receptor (GR) in addition to steroid sulfatase (STS) and 17 β hydroxysteroid dehydrogenase 2 (17 β HSD2). We also evaluated immunoreactivity of Ki-67 and CYP19/aromatase. We correlated these factors with the occurrence of distant metastasis or local relapse, and survival adjusted by age at surgery. We also examined whether any of these pathways were enriched in the classical breast cancer subtypes (Luminal A, Luminal B, HER-2 positive, triple-negative BC). Logistic and Cox regression analysis was employed to examine interactions between the above factors.

Results: In an analysis looking at possible interactions between recurrence and the factors above, the status of intratumoral STS was significantly inversely associated with distant metastasis (OR=0.17, $p<0.001$) and local relapses (OR=0.17, $p<0.001$), while that of 17 β HSD2 trended towards correlation with the presence of metastasis (OR=3.47, $p=0.055$) and relapse (OR=3.14, $p=0.07$). Interestingly, ER and PR were not correlated with metastasis or relapse, but HER2 status positively correlated with distant metastasis during follow-up (OR=2.15, $p=0.02$) and trended to be associated with local relapses as well (OR=1.99, $p=0.054$). When evaluated according to the established breast cancer subtypes the only significant changes detected between subgroups were for AR and STS. AR was positive in a lesser proportion of the cases in the TNBC subgrouping ($p=0.01$), while STS was depleted in the luminal B and enriched in the HER2 subtypes ($p=0.027$). In an analysis of survival, STS was the only factor examined that was significantly associated with survival outcomes of the patients. Positive intratumoral STS status was significantly associated with improved survival (HR=0.27, $p<0.001$). The inverse of this was also noticed for 17 β HSD2 but the correlation did not reach statistical significance (HR=2.1, $p=0.16$).

Discussion and Conclusions: The results of our present study suggest that the intratumoral metabolism of estrogens through STS is associated with significantly lower incidence of relapse and/or distant metastasis and correspondingly a better prognosis. The inverse is also true when evaluating the trends noted with the expression of 17 β HSD2 adding consistency to our finding above. The enrichment of STS in the HER2 overexpressing subtype of breast cancer is intriguing, especially given the possible role of HER-2 positivity in endocrine resistance. All in all, our results suggest that extended endocrine information about the intratumoral steroid metabolism of a given tumour may have so far unused prognostic potential and impact on clinical decision making.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-05-09

Title: Glucocorticoid receptor modulation affects ER+ breast cancer cell proliferation

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Body: Early-stage ER+ breast cancer with high tumor glucocorticoid receptor (GR) expression is associated with improved long term relapse-free survival compared to tumors with low GR expression. In addition, activation of GR inhibits estradiol-mediated ER+ BC cell proliferation. This finding led us to hypothesize that GR and ER engage in nuclear receptor cross-talk to affect pro-proliferative gene expression, thus contributing to a better outcome in ER+/GR+ BC patients. To better understand the mechanisms by which ER/GR co-activation contributes to this more indolent phenotype, we performed ChIP-sequencing and gene expression analyses in ER+/GR+ BC cell lines (MCF-7 and T47D).

We found that co-activation of ER and GR led to decreased ER+ BC cell proliferation *in vitro*. Furthermore, following co-activation of ER/GR, there was decreased gene expression of key cell cycle genes (e.g. *CDK6*, *CDK2* and *CCNE1*) compared to ER-activation alone. Studies are underway to determine if this decrease in gene expression is associated with less CDK activity as well as a slowing of cell cycle progression.

We also wanted to know if GR activation with a pure agonist versus a mixed GR modulator was required for decreasing ER-mediated cell proliferation. Based on our previous work (DC West *et al. MCR* 2016), we hypothesized that ligand-mediated GR activation could be working by preventing access of ER to regulatory regions of pro-proliferative genes rather than causing direct GR agonist-mediated gene expression. To examine this question, we used a GR modulator, mifepristone (expected to alter ER-mediated gene expression indirectly). Indeed we found that a GR modulator also reduced ER-mediated cell proliferation. We are currently testing the mechanisms by which GR modulators affect estradiol-mediated cell cycle gene expression.

Taken together, these studies suggest that modulation of GR activity could be an effective approach to decrease ER+ tumor cell proliferation. Xenograft studies are underway to examine whether the addition of GR modulation to tamoxifen will improve tumor shrinkage compared to tamoxifen alone.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-05-10

Title: Evaluation of HER2 expression and amplification on CTCs using DEPAarray analysis and sorting followed by FISH

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Body: Introduction

In metastatic breast cancer (MBC), discordant expression levels of the human epidermal growth factor receptor 2 (HER2) have been noted between primary tumors (PT) and matched metastatic lesions. Reassessment of HER2 status during treatment decisions in patients with advanced disease might help to optimize outcome. Circulating tumor cells (CTCs) offer the potential to provide a repeatedly accessible source of tumor cells for the real-time assessment of actual tumor characteristics. However, little is known on the concordance of HER2 expression on CTCs measured by immunofluorescence and the amplification status. Here we report on a preclinical study, using five spiked breast cancer cell lines, comparing semi-quantitative HER2 scoring on CellSearch (Riethdorf 2010) with objective DEPAarray analysis, and subsequent FISH analysis on DEPAarray-sorted tumor cells. At the moment these data are also being generated for 10 patient CTC samples. Expression and amplification status of CTCs will be compared with primary tumor tissue.

Materials and methods

MDA-MB-436, MCF-7, BT-20, KPL-4, and SKBR3 cells (increasing HER2 status) were spiked into donor blood and subjected to CellSearch enrichment. HER2/FITC intensity was scored manually on the CellSearch analyzer. All cell lines were injected into the DEPAarray and exposure settings were optimized (FITC: exposure time 800 ms, gain 5%). These settings are further used for all preclinical and clinical samples. HER2 scoring was based on relative fluorescent units (rfu) of the HER2/FITC signal with background subtraction. Cells were sorted into pure batches of HER2 positive (DAPI+/CK+/HER2+/CD45-) and negative (DAPI+/CK+/HER2-/CD45-) tumor cells. Cytospins were formalin fixed and subjected to DAKO IQFISH.

Results

HER2 expression on CellSearch turned out to be very heterogeneous within the same cell line. DEPAarray data was highly reproducible for all cell lines ($p < 0.001$) and also showed a broad range of FITC rfu within the HER2 positive cell lines. Significant differences were observed between every cell line ($p < 0.001$). The SKBR3 cell line sample also harbored a minor population of HER2- cells while this was the most positive cell line. However with FISH analysis, both HER2- and HER2+ SKBR3 cells were highly amplified (absolute HER2 count of 12-20 and HER2/CEN17 ratio of >4). MDA-MB-436 and MCF-7 cells showed no gene amplification on FISH, while in KPL-4 there was a HER2/CEN17 ratio of >2 .

Four patient samples with HER2 positive status on CellSearch have been run on the DEPAarray. For patient 1, 1005 CTC were analyzed, 32.4% were HER2+. This was 53 (69.8% HER2+), 352 (5.7% HER2+), and 622 (6.7% HER2+) for patient 2-4 respectively. These numbers are comparable with CellSearch analysis.

Discussion

HER2 expression analysis by immunofluorescence is comparable between CellSearch and DEPAarray, however DEPAarray has the advantage that it is user-independent and highly reproducible. Furthermore, CTCs can be sorted into pure batches for downstream analysis. The FISH technique on DEPAarray sorted cells is now optimized and will be used to determine the correlation between the immunofluorescent HER2 scoring and the actual amplification status of the CTCs. These data will be incorporated prior to upcoming SABCS.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-05-11

Title: Clinical outcomes of estrogen-receptor negative breast carcinoma are associated with protein hormone-cognate receptor gene expression

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Body: Background: Since certain protein hormones appear to be associated with progression of breast cancer cells *in vitro*, we employed a global approach by evaluating relationships between genes for these hormones and their cognate receptors in human breast cancer biopsies as independent predictors of risk of recurrence. Our goal is to derive clinically relevant molecular signatures correlating various ER/PR subtypes.

Methods: Microarray analyses of LCM-procured carcinoma cells from 247 de-identified biopsies determined expression of genes for 61 protein hormones (the ligands) and 81 cognate receptors. Total RNA was extracted, purified and amplified from cells to determine expression of 22,000 genes. Univariable and multivariable Cox regressions were determined using expression levels of each hormone/receptor gene. Kaplan-Meier plots were constructed with an adjusted p-value <0.30 (discovery cutoff) for each gene candidates with/without ER status of the lesion. Cox regression analyses with/without interaction models deciphered candidates of hormone-receptor complexes that predicted risk of recurrence as well as overall survival using clinical follow-up that extended as much as 12 years. LASSO was used to derive gene subsets to predict overall survival (OS) and progression free survival (PFS) within ER/PR subtypes.

Results: Expression levels of 7 genes for protein hormones and 10 receptor genes were significant for OS at an adjusted p-value of <0.30 while expression of 15 hormone and 19 receptor genes were significant for PFS without regard to ER/PR subtypes. When expression levels were considered with ER status only, 3 genes for ER+ lesions and 6 genes for ER- lesions were identified that predicted OS/PFS. Categorization of cancers according to combined ER and PR status revealed one gene for ER+/PR+, 3 genes for ER+/PR- and 2 genes for ER-/PR- associated with PFS/OS. After stratifying gene expression into above/below median for the linear predictor, Kaplan-Meier plots revealed patient groups with each signature for ER+/PR+, ER-/PR- and ER- lesions that exhibited significantly different survival. Molecular signatures comprising 16 genes for ER+/PR+ carcinomas, 10 genes for ER-/PR- lesions and 6 genes for ER- carcinomas predicted clinical outcomes with C-indices of 0.79, 0.76 and 0.71, respectively.

Conclusion: We revealed many breast carcinomas synthesize mRNA species for various protein hormones and their cognate receptors by determining gene expression directly on pure populations of these cells procured by LCM. We report a novel ten-gene ER-/PR- signature containing four genes in common with that of a six-gene ER- only signature that predicts breast cancer recurrence. Collectively, results of mRNA expression suggest that often breast carcinomas exhibit substantial elements of endocrine autonomy for regulating progression, warranting investigation of protein products of gene candidates in isolated populations of breast carcinoma cells.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-06-01

Title: Therapeutic targeting of RAGE in the tumor and tumor microenvironment inhibits breast progression and metastasis

Kwak T, Drews-Elger K, Ergonul A, Braley A, Hwang GH H, El-Ashry D, Slingerland JM M, Lippman ME E and Hudson BI I.
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Body: Background: The Receptor for Advanced-Glycation End-products (RAGE) is highly expressed in various cancers and its expression is correlated with poorer outcomes in breast cancer. We have previously implicated RAGE in breast cancer, but whether RAGE drives breast cancer progression and metastasis either through tumor cell intrinsic effects, non-tumor cells of the tumor microenvironment, or both, is not fully understood. More importantly, studies are lacking that target RAGE therapeutically in cancer, and may therefore represent a novel treatment for breast cancer metastasis.

Methods: Using multiple human and murine breast cancer models we dissected the tumor intrinsic versus tumor microenvironment role of RAGE in metastasis. RAGE was targeted in tumor cells using multiple shRNAs, in non-tumor cells by global gene knockout in mice, and both by therapeutically targeting with the novel RAGE inhibitor FPS-ZM1. In vivo orthotopic models included the NSG (NOD-SCID-gamma) xenograft mouse model (with MDA-MB-231 cells; herein 231), BALBc (4T-1 and 67NR), and C57BL6 wild-type and RAGE knockout (RAGE $-/-$) mice (with MMTV-PyMT spontaneous breast cancer derived AT-3 cells).

Results: We first tested how RAGE impacts tumor cell intrinsic mechanisms using either *RAGE* shRNAs or FPS-ZM1 in 231, 4175 (231 isogenic highly metastatic cells) and 4T-1 cells. *RAGE* shRNA and FPS-ZM1 both decreased RAGE MAP-kinase signaling, transwell invasion and soft agar colony formation, without affecting proliferation. In vivo, *RAGE* shRNA knockdown in 231 cells did not affect tumor growth, but inhibited metastasis to lung and liver. *RAGE* shRNA knockdown in 4175 cells, decreased orthotopic tumor growth, and reduced tumor angiogenesis and tumor recruitment of leukocyte / macrophages. Furthermore, *RAGE* shRNA knockdown dramatically decreased metastasis of 4175 cells to lung and liver in a time and sized matched manner compared to shRNA controls. Similarly, RAGE knockdown in 4T-1 cells reduced cell invasion and colony formation, and inhibited lung metastasis from the orthotopic site in BALBc immunocompetent mice.

To test the non-tumor cell microenvironment role of RAGE, we performed syngeneic studies with orthotopically injected AT-3 cells in RAGE $+/+$ and RAGE $-/-$ C57BL6 mice. RAGE $-/-$ mice displayed striking impairment of tumor cell growth compared to RAGE $+/+$ mice, along with decreased MAP-kinase signaling, tumor angiogenesis and inflammatory cell recruitment.

Finally, to test the combined inhibition of RAGE in both tumor cell intrinsic and non-tumor cells of the microenvironment, we performed in vivo treatment of 4175 tumors with FPS-ZM1 (1mg/kg, twice per week). Compared to vehicle, FPS-ZM1 inhibited primary tumor growth, inhibited tumor angiogenesis and inflammatory cell recruitment, and most importantly prevented metastasis to lung and liver.

Conclusion: These data clearly demonstrate a role for RAGE in breast cancer progression and metastasis through distinct effects in the tumor cell and non-tumor cells of the tumor microenvironment. Furthermore, our data from drug inhibitor studies highlight the combined targeting of RAGE in the tumor and tumor microenvironment, and as a viable therapeutic means for breast and other metastatic cancers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-06-02

Title: ONC201 kills breast cancer cells by inhibiting mitochondrial respiration

Greer YE E, Gilbert SF F, Islam C, Ji Y, Gattinoni L, Stuelten C, Voeller D and Lipkowitz S. Women's Malignancies Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD; Experimental Transplantation and Immunology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD and Laboratory of Cellular and Molecular Biology, Center for Cancer Research, National Cancer Institute, Bethesda, MD.

Body: Background: ONC201 is a small molecule originally identified as a TRAIL inducing compound (Allen et al., *Sci. Trans. Med* 2013). Two recent studies reported that ONC201 also induces an atypical stress response mediated in part by ATF4 and CHOP (Klein et al., *Sci. Signal* 2016 and Ishizawa et al., *Sci. Signal* 2016). ONC201 is currently being tested in phase 1/2 clinical trials in multiple cancer types. In this study, we tested the effects of ONC201 on human breast cancer cells.

Methods: We tested ONC201 in 18 human breast cancer cell lines that represent ER+, HER2 amplified, TNBC basal A and TNBC basal B breast cancer. Cell death was analyzed by MTS assay after 5 days of treatment. Cells were treated with GST-TRAIL in parallel for comparison. Z-VAD-FMK was used as a pan-caspase inhibitor. To verify the mechanism of action of ONC201, siRNA against death receptors (DR) 4 and 5 were transfected to cells and tested in MTS assay and Western blotting. Seahorse XF analyzer and live cell imaging were used to further characterize the effect of ONC201.

Results: ONC201 reduced cell viability in breast cancer cell lines in all subtypes tested with IC50s ranging from 0.8-5 μ M, similar to what has been reported for other cancer cell types. Unexpectedly, ONC201 did not induce caspase 3 or PARP cleavage, and its toxicity was not inhibited by Z-VAD-FMK, nor by siRNA knockdown of DR4 or DR5. By contrast GST-TRAIL induced caspase 3 and PARP cleavage and GST-TRAIL-induced cell death was inhibited by Z-VAD-FMK and by siRNA knockdown of DR5. Live cell imaging revealed ONC201 induces cell membrane ballooning followed by rupture, whereas GST-TRAIL induced classic apoptosis morphology. Together these results suggest that ONC201 kills breast cancer cells via a caspase-independent, DR4/5-independent mechanism distinct from TRAIL-induced apoptosis. Western blots revealed that ONC201 induces ATF4 and CHOP, consistent with the recently published observations. ONC201 also induced phosphorylation of AMP-dependent kinase (AMPK) in multiple breast cancer cell lines, suggesting that cellular ATP level is decreased by ONC201. ATP depletion by ONC201 was confirmed by direct measurement of cellular ATP. Seahorse XF analysis found that ONC201 inhibited mitochondrial oxygen consumption rate (OCR) but did not inhibit glycolysis as measured by the extracellular acidification rate. Long exposure to ONC201 significantly attenuated OCR, while acute treatment did not inhibit OCR. These data suggest that ONC201 inhibits mitochondrial oxidative phosphorylation via an indirect mechanism. Western blots demonstrated that ONC201 decreases expression of multiple mitochondrial proteins involved in oxidative phosphorylation. Both ONC201-induced toxicity and ATP depletion were enhanced when cells were cultured in non-glucose (galactose) medium. This is consistent with ONC201-induced inhibition of mitochondrial respiration. Supplementing glucose to cells grown in galactose medium partially rescued ONC201-dependent ATP depletion and cell death, and reversed ONC201-induced phospho-eIF2, ATF4 and CHOP induction.

Conclusion: Together, these data demonstrate that ONC201 can kill breast cancer cells by a novel mechanism involving inhibition of mitochondrial respiration.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-06-03

Title: The short term effects of an AKT inhibitor (AZD5363) on biomarkers of the AKT pathway and anti-tumour activity in a breast cancer paired biopsy study (STAKT trial)

Roberston JFR FR, Cheung KL, Ahmed S, Coleman RE, Evans A, Holcombe C, Rea D, Rauchhaus P, Skene A, Littleford R, Jahan A, Kelly S, Lindermann JPO PO, Horgan K, Foxley A, Rugman P and Pass M. University of Nottingham, Nottingham, United Kingdom; Univeristy of Dundee, Dundee, United Kingdom; University of Sheffield, Sheffield, United Kingdom; Royal Liverpool University Hospital, Liverpool, United Kingdom; King's Mill Hospital, Nottingham, United Kingdom; Leeds General Infirmary, Leeds, United Kingdom; Weston Park Hospital, Sheffield, United Kingdom; Leicester Royal Infirmary, Leicester, United Kingdom; Royal Bournemouth & Christchurch NHS Foundation, Bournemouth, United Kingdom; Poole Hospital NHS Foundation Trust, Poole, United Kingdom; Royal Derby Hospital, Derby, United Kingdom; University of Birmingham, Birmingham, United Kingdom; Derriford Hospital, Plymouth, United Kingdom and AstraZeneca, Melbourn, United Kingdom.

Body: Background: AKT is an important intracellular control point through which Type 1 growth factors (HER family) and IGFR signalling from the membrane exert their influence on tumours, including estrogen receptor positive (ER+) breast cancer. Mutations in *PIK3CA*, *AKT* and *PTEN* are prevalent in breast cancer and can directly impact on AKT signalling. AKT has also been implicated in resistance to signal transduction inhibitors, including endocrine therapies. AZD5363 is an inhibitor of AKT 1, 2 and 3 which is in Phase 2 trials for breast and other cancers.

Design: The study was designed to address the extent to which AZD5363 impacts key biomarkers on the AKT pathway, and the degree to which those impacts translate to effects on markers of anti-tumour efficacy (Ki67) in ER+ breast cancer.

STAKT is a multicentre, two-stage, double blind, randomised, placebo controlled, biomarker window trial conducted in women with ER+ breast cancer. Stage 1 assessed the effect of AZD5363 at a dose of 480mg bd p.o. versus matching placebo. Up to 30 patients per arm were allowed in order to reach 24 evaluable patients (12 per study arm) with assessable paired biopsies. Tumour biopsies were obtained at baseline, and following 4.5 days of AZD5363 / placebo exposure. The primary endpoint markers were pPRAS40, pGSK3b and Ki67. Biomarker assessment was performed by immunohistochemistry by laboratory staff blinded to treatment arm and also whether the biopsy was from baseline or post AZD5363/placebo. PK sampling was performed during the study period.

Stage 2 is ongoing with the focus on the biomarker effects of lower doses of AZD5363.

Results:

35 patients were recruited to Stage 1 with 24 patients established as evaluable. Patient characteristics and demographics are shown in Table (to be updated prior to abstract review).

Un-blinded data from Stage 1 will show the changes in each of the primary endpoints (pPRAS40, pGSK3b and Ki67) for AZD5363 480 bd mg versus placebo, the pharmacokinetics and the adverse event profile of AZD5363.

Conclusions

This study will report short-term biomarker and pharmacokinetic data with AZD5363 in women with early breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-06-04

Title: Modification and reversal of the aggressive behavior of triple negative breast cancer by caffeic acid phenethyl ester (CAPE)

Omene C, Patel M, Illa Bochaca I and Barcellos-Hoff MH. NYU Langone Medical Center, NYU School of Medicine, New York, NY and University of California, San Francisco, San Francisco, CA.

Body: CAPE is the major active component of propolis, a widely available, safe, honeybee natural product with anti-inflammatory, antioxidant, and antitumor properties. We have previously shown diverse effects of CAPE in breast cancer. We postulated that CAPE may be useful in chemoprevention for women at high risk for triple-negative breast cancers (TNBC) and evaluated this in a radiation chimera mouse model in which the tumor spectrum is shifted to TNBC.

The radiation chimera model consists of surgically clearing the mammary epithelium from the inguinal glands of 3-week old BALB/c mice, the mice were irradiated with 1 Gy or sham irradiated at 10–12 weeks of age, and bilaterally transplanted 3 days later with syngeneic *Trp53* null mammary fragments. Mice were placed on a CAPE diet or a control diet at 1 month post transplantation that was maintained for the course of the experiment. Mammary tumor development was monitored by palpation for up to 18 months. The first tumor was resected at 1cm³ and mice monitored for tumor recurrence or second tumor formation. Tumors were immunostained for estrogen receptor (ER) status and ER negative tumors were selected from all the groups for RNA sequencing.

No difference in body weight or tumor incidence was observed between mice on the CAPE versus control diet. Host irradiation significantly accelerated tumor growth rate compared to the control sham irradiated mice. CAPE treatment blocked this effect, but did not affect the control tumor growth rate. Resected tumors in CAPE treated mice recurred at significantly longer intervals (average of 40 days in the control group versus 90 days with CAPE treatment) and much less frequently than tumors from mice on a control diet. Mean expression analysis showed that CAPE induces a distinct gene expression pattern in ER negative tumors from irradiated mice. As previously described, the transcriptional profile of ER negative tumors was enriched in immune response genes in tumors from nonirradiated mice on a control diet, while irradiation suppresses these genes. Interestingly, this difference was abrogated in mice on the CAPE diet where we found that CAPE reverses the suppression of immune response genes in the ER negative tumors from the irradiated mice.

These findings support the potential use of CAPE to modify the aggressive behavior of TNBC. This may be due to effects on the immune system in which CAPE acts to re-establish anti tumor immunity. Thus, CAPE may be useful in chemoprevention both for women at high risk for TNBC and to prevent or delay TNBC breast cancer recurrence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-06-05

Title: Receptor-mediated binding of HER2-targeted antibody-liposomal doxorubicin conjugate MM-302 increases liposome binding, nuclear doxorubicin, DNA damage and efficacy relative to untargeted PEGylated liposomal doxorubicin (PLD/Doxil)

Espelin C, Geretti E, Coma S, Koncki Z, Leonard S, Dumont N, Reynolds J, Molnar I and Wickham T. Merrimack Pharmaceuticals Inc., Cambridge, MA.

Body: Background: Liposomal encapsulation of doxorubicin increases drug payload, extends circulation time (PK/PD) resulting in increased tumor accumulation and reduces systemic toxicity, notably cardiotoxicity. Once in the tumor microenvironment, targeting moieties provide a means to enhance liposome uptake by tumor cells overexpressing the desired target. MM-302 is a HER2-targeted antibody–liposomal doxorubicin conjugate designed to specifically deliver doxorubicin to HER2-expressing tumor cells. HER2 overexpression (“HER2-positive”) is detected in 20-25% of breast cancer tumors and associated with a particularly aggressive subtype of the disease. MM-302 demonstrated an acceptable safety profile and promising activity in a Phase I study in HER2-positive metastatic breast cancer (NCT01304797) and is now being evaluated in a Phase II trial in the same setting (NCT02213744).

Methods: A panel of cell lines representing a range of HER2 levels from “0” to “3+” were treated *in vitro* with MM-302 or untargeted PLD. Bound liposome, nuclear doxorubicin, DNA damage response (γ H2AX, p-p53, CHK2), apoptosis (cl. CASP3 and cl. PARP), secreted cytokines and cell viability were evaluated with a combination of high throughput microscopy, Meso Scale Discovery (MSD) assay, ELISA and CTG assay. *In vivo*, single cell HER2 levels were determined by IHC for multiple human xenograft models. Additionally, xenograft tumors treated with MM-302 or PLD were dissociated and single cell binding of liposome was evaluated by FACS while p-p53, p-CHK2 and cytokine levels were tested by MSD/ELISA. Changes in tumor volume of BT474-M3 and SUM-190 breast cancer xenografts treated with MM-302 or PLD were determined.

Results: Cells expressing ~150K to 2 million HER2 receptors/cell demonstrated preferential binding of MM-302 and subsequent accumulation of nuclear doxorubicin, relative to PLD. Increased DNA Damage Response (γ H2AX, p-p53, p-CHK2), apoptosis (cleaved PARP, cleaved Caspase 3) and cell death correlated with bound MM-302 *in vitro*. Levels of the secreted cytokines IL-6 and IL-8 were associated with bound MM-302, but not PLD. *In vivo*, cell-bound MM-302 and DNA-damage response (p-p53/p-CHK2) increased with HER2 level while bound PLD was consistently low, regardless of HER2. MM-302 significantly reduced BT474-M3 and SUM-190 tumor volume relative to PLD.

Conclusions: MM-302 was bound by all cell lines expressing HER2 to a significantly greater extent than PLD, with resultant delivery of doxorubicin to nucleus, DNA-damage, and ultimately cell death. The benefit of HER2-targeting by MM-302 is also evident *in vivo* at the single-cell level and manifests itself in greater tumor reduction.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-06-06

Title: Development of iNPG-pDox for metastatic breast cancer treatment

Ferrari M, Xu R, Zhang G, Mai J and Shen H. Houston Methodist Research Institute, Houston, TX and Huazhong University of Science and Technology, Wuhan, China.

Body: Metastatic triple negative breast cancers have traditionally been treated with chemotherapy. However, these drugs have narrow therapeutic windows due to low drug accumulation in the tumor tissue, poor pharmacokinetic profile, and severe toxicity to the normal tissues. Repeated treatments at sub-optimal dosages are counter-effective and too often lead to therapy resistance. We have developed an injectable nanoparticle generator (iNPG) that overcomes multiple biological barriers to cancer drug delivery (Xu et al. *Nature Biotechnology* 2016; 34: 414-418). The iNPG is a discoidal micrometer-size particle that can be loaded with chemotherapeutics. We conjugated doxorubicin to poly(L-glutamic acid) via a pH-sensitive cleavable linker, and load the polymeric drug (pDox) into iNPG to assemble iNPG-pDox. Once released from iNPG, pDox aggregates spontaneously into nanoparticles in an aqueous solution. The intravenously injected iNPG-pDox accumulates at tumors and releases pDox nanoparticles that are internalized by tumor cells. Inside the cell, pDox nanoparticles are transported to the perinuclear region and cleaved into Dox, thereby avoiding excretion by drug efflux pumps. We compared therapeutic efficacy between iNPG-pDox and the current clinic anthracycline drugs in mouse models of metastatic MDA-MB-231 and 4T1 tumors. While the current drugs offered marginal survival benefit, treatment with an equivalent dosage (normalized based on doxorubicin content) of iNPG-pDox significantly extended animal survival, including long-term survival in 40% of the 4T1 tumor mice and 50% of the MDA-MB-231 tumor mice. This result demonstrates the power of incorporating concurrent actions in drug design including drug enrichment in the tumor tissue, *in situ* reconstitution of drug nanoparticle, vesicular transport to circumvent the efflux pumps, and pH-dependent drug release.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-06-07

Title: Combination of doxorubicin with S1P signaling modulator FTY720 significantly suppressed obesity-associated breast cancer

Katsuta E and Takabe K. Breast Surgery, Roswell Park Cancer Institute, Buffalo, NY.

Body: Background:

Obesity is one of the biggest health issues in the US. It has been shown that obesity-associated breast cancer is more aggressive with poor prognosis, which is partly explained by the low-grade inflammation caused by obesity. Recently we have published that sphingosine-1-phosphate (S1P), a signaling lipid mediator, link inflammation and cancer in colitis-associated colon cancer model. We hypothesized that addition of S1P modulator that block S1P signaling thus suppress the effect of obesity-mediated inflammation should enhance anti-cancer effect of doxorubicin, which is a typical anti-cancer drug for breast cancer used as a standard of care.

Methods:

Female B6.cg-*Lep*^{ob} (OB/OB) mice fed with high fat diet for 2 weeks prior to implantation of cancer cells were used as an obesity model, and litter mate control mice fed with normal diet were used as a control. 1×10^6 murine mammary adenocarcinoma E0771 cells were inoculated into #2 rt. fat pads as previously described (Katsuta et al JSR 2016). 9 days after inoculation, both OB/OB and control mice were randomized into 4 groups in each group; vehicle, Doxorubicin, FTY720 and Combination of Doxorubicin and FTY720. Doxorubicin was administrated by i.p. injection at a dose of 5 mg/kg on Day 0 and 3. FTY720 was administered everyday by gavage at a dose of 1 mg/kg during the entire course. Tumor growths were measured daily by caliper measurements. Tumor weights were measured on 21 days after cell inoculation.

Results:

The body weight of obesity model was significantly heavier than control mice at the time of cancer cell inoculation (44.1 g vs 19.4 g; $p < 0.001$). In non-treatment group, tumor weight in obesity group was significant heavier than control mice[KT1] (1232 mg vs 966 mg; $p = 0.049$), which is consistent with the dogma that obesity worsen cancer progression. As expected, tumor weight in non-treatment group is heavier than any treatment group, and that in combination treatment of doxorubicin and FTY720 is lightest in both of obesity group and control group. Interestingly, tumor reduction rate in obesity group compared with non-treatment group is significant greater than control group (Doxorubicin: 83% vs 19%, $p = 0.001$; FTY720: 80% vs 46%, $p = 0.027$, Doxorubicin + FTY720: 93% vs 64%, $p = 0.011$). Over 15 % weight loss were seen in obesity doxorubicin group and obesity combination treatment group.

Conclusion:

Modification of S1P signaling by FTY720 was shown to enhance the effect of doxorubicin particularly in obese mice, which implicate a novel approach to treat obesity-associated breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-06-08

Title: HDAC6 deacetylates HMGN2 to regulate Stat5a activity and breast cancer growth

Clevenger CV V, Craig JM M, Fiorillo AA A, Feeney YB B, Harrell JC Chuck and Medler TR R. Virginia Commonwealth University, Richmond, VA; George Washington University, Washington, DC; Northwestern University, Chicago, IL and Oregon Health Sciences University, Portland, OR.

Body: Stat5 is a transcription factor utilized by several cytokine/hormone receptor signaling pathways that promotes transcription of genes associated with proliferation, differentiation, and survival of cancer cells. However, there are currently no clinically approved therapies that target Stat5, despite ample evidence that it contributes to breast cancer pathogenesis. Previous research in our lab has shown that the high mobility group nucleosome binding domain 2 (HMGN2) protein serves as a Stat5 co-activator. The activity of HMGN2 has been previously shown to be regulated by acetylation. Here, we show that deacetylation of HMGN2 on lysine residue K2 by histone deacetylase 6 (HDAC6) promotes Stat5-mediated transcription and breast cancer growth. Conversely, in vitro HDAC6 inhibition by pharmacologic and knock-down approaches enhanced HMGN2 acetylation with a concomitant reduction of in vitro Stat5-mediated signaling and global gene expression, and breast cancer growth. In vitro and in vivo treatment of traditional and patient-derived xenograft models with HDAC6 inhibitors resulted in a highly significant reduction of tumor growth. Translationally, it was also found that high levels of acetylated K2 were present in normal human breast tissue, which was lost in primary breast cancers and lymph node metastases. This suggests that blockade of HMGN2 deacetylation as described above is a novel treatment for breast cancer, given that existing HDAC6 inhibitors have a favorable toxicity profile in Phase I trials of other tumor types. Altogether, these results reveal a novel mechanism through which HDAC6 regulates the transcription of Stat5 target genes and demonstrates the utility of HDAC6 inhibition as a potential breast cancer therapeutic.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-06-09

Title: Selection of presumed CD49f antagonists and their biological evaluation in breast cancer cells

Velasco-Velázquez M, Velázquez-Quesada I, Aguirre-Alvarado C, Guerrero-Rodríguez S, Ruiz-Moreno A, Ramirez-Salinas G, Segura-Cabrera A and Pérez-Tapia M. School of Medicine, National Autonomous University of Mexico, Mexico City, Mexico; National Polytechnic Institute, Mexico City, Mexico; CONACYT, Mexico City, Mexico and Institute of Ecology AC, Xalapa, Veracruz, Mexico.

Body: Breast cancer is the second cause of cancer death in women and the large majority of those deaths are due to drug resistance and/or recurrence. The cancer stem cell (CSC) model suggests that tumor stem-like cells play key roles in resistance and recurrence in breast cancer patients. Thus, CSCs have been pointed as targets for new anti-cancer therapies. CD49f is an integrin subunit that participates in the CSC-niche interaction. Knock-down of CD49f in breast cancer cells impairs mammosphere formation *in vitro* and tumorigenesis *in vivo*, suggesting that this protein is needed for maintenance of stemness.

Aiming to target breast CSC, we used consensus docking to select potential CD49f antagonists from a collection of 13,000+ drugs with previous clinical evaluations. Seven compounds with the lowest consensus Z score were selected for *in vitro* biological validation. Cell adhesion assays showed that four of the selected drugs (5193, 1382, 7631, and 12723) decrease the binding of CD49f+ MDA-MB-231 breast cancer cells to laminin, suggesting that those compounds antagonize the receptor. Mammosphere formation assays showed that compounds 5193, 1382, and 12723 limit the clonogenic capability of CD49f+ breast cancer cells *in vitro*. Compound 5193 was highly toxic in 2D cell viability assays. On the other hand, the clonogenic impairment produced by compounds 1382 and 12723 is independent of bulk cell line cytotoxicity, suggesting a direct impact on the CSC pool. The analyses of the effect of these drugs on the expression of breast CSC markers and CD49f-activated pathways are on their way. Thus, we have identified some drugs that might be repurposed to target breast CSC. Since the pharmacokinetics and toxicology of those drugs is known, they could *easily* be ready for clinical trials. We demonstrated that *in silico* screening is useful to identify antagonists of receptors with relevant roles in breast CSC biology.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-01

Title: Selinexor, a selective inhibitor of nuclear export, demonstrates efficacy in preclinical models of triple negative breast cancer

Paez Arango N, Evans KW W, Zhao M, Yuca E, Scott SM M, Janku F, Ueno NT T, Tripathy D, Kim C, Naing A and Funda M-B.
MD Anderson Cancer Center, Houston, TX.

Body: Background: Approximately 15% of all breast cancers are categorized as triple negative (TNBC) for which the only chemotherapy is known to be effective, yet often fails to achieve remission. Nuclear exporter XPO1 (Exportin1 or CRM1) is a promising target for cancer therapy that mediates the transport of multiple tumor suppressors and cell cycle regulators that have been known to be relevant predictors in the mechanism and severity of TNBC. Given the pressing need for novel therapies for this disease, we sought to determine the antitumor effects of selinexor, a novel inhibitor of nuclear export, on triple negative breast cancers *in vitro* and *in vivo* as well as to address its mechanism of action.

Methods: 26 breast cancer cell lines of different breast cancer subtypes were treated with selinexor *in vitro*. Using cell proliferation assays the half maximal inhibitory concentration (IC₅₀) was calculated using isobologram curves after 3 days of treatment; sensitivity was defined as IC₅₀ <1000nM. We then assessed mechanistic effects on apoptosis and cell proliferation using flow cytometry analysis with annexin V and propidium iodide and using western blot analysis we also studied its effects on markers of inhibition of apoptosis. *In vivo* efficacy was studied as single agent and in combination with standard chemotherapy agents in TNBC patient derived xenografts (PDXs) with varying levels of sensitivity to chemotherapy as well as with varying statuses of TP53 and PIK3CA, and gene expression subtypes.

Results: Selinexor demonstrated growth inhibition in all fourteen TNBC cell lines tested; TNBC cell lines were more sensitive to selinexor (median IC₅₀ 44nM, range 11 - 550nM), compared to ER+ cells lines (median IC₅₀ of 13000 nM, range of 40nM - > 1000 nM; P=0.017). Treatment with selinexor decreased expression levels of XPO1, as well as survivin and XIAP, and induced apoptosis. In multiple TNBC cell lines selinexor was synergistic with paclitaxel, carboplatin, eribulin and doxorubicin *in vitro* (median combination index 0.6, range 0.5-0.8). Selinexor as a single agent reduced tumor growth *in vivo* in 4 of 5 different TNBC PDX models with a median tumor growth inhibition ratio score (T/C) of 48% (range 34-59%) and demonstrated greater antitumor efficacy in combination with paclitaxel or eribulin with an average T/C score of 27% and 12% respectively.

Conclusions: Selinexor is a promising therapeutic agent for triple negative breast cancer and it has potential as a combination agent with standard chemotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-02

Title: Are we missing actionable targets in breast cancer? Novel insights into recurrent Ret alterations

Hirshfield KM M, Paratala BS S, Hindoyan A, Dolfi SC C, Yilmazel B, Schrock A, Gay L, Ali SM M, Ross JS S, Williams CB B, Nair P, Ganesan S and Leyland-Jones B. Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; Molecular Response LLC, San Diego, CA; Foundation Medicine, Cambridge, MA and Avera Center for Precision Oncology, Sioux Falls, SD.

Body: Background: Recurrent gene fusions in breast cancer have been rarely reported suggesting that they either are not present or are not easily detected by standard sequencing methods. Comprehensive genomic profiling (CGP) by hybrid capture-based, high depth next-generation sequencing approaches, can be used to detect recurrent rearrangements and other genomic alterations involving target genes. We found that CGP can identify recurrent alterations involving RET, a known oncogenic tyrosine receptor kinase, in a subset of breast cancer.

Methods: CGP using FoundationOne platform was performed interrogating the entire coding region for up to 315 cancer-related genes and introns of up to 28 genes involved in rearrangements at a depth of 500-1000X in formalin-fixed, paraffin embedded tumor tissue (Foundation Medicine, MA). Engineered representative RET fusion vectors were synthesized and expressed in non-tumorigenic cell lines (breast MCF10A and mouse 3T3 fibroblasts), and cells were evaluated for RET kinase signaling, drug response, and tumorigenicity. Patient-derived xenografts (PDX) generated from two triple negative breast cancers (TNBCs) were used in an ex vivo assay (Response3DX™, Molecular Response LLC, San Diego, CA).

Results: Twenty-two RET rearrangements were identified in 8119 (0.27%) breast cancer cases. Of these, 5 rearrangements were activating RET fusions including CCDC6-RET (n=4) and NCOA4-RET (n=1), that have been described in other cancer types. Five other cases had clear evidence of genomic rearrangement involving RET, but the 5' partners could not be definitively identified. The remaining twelve cases had complex rearrangements of RET including internal duplications. RET amplification was also observed, both in TNBC and in a HER2+ breast cancer at onset of resistance to HER2-targeted therapy.

Both NCOA4-RET and a novel RASGEF1A-RET fusion were characterized in vitro. Non-tumorigenic cells engineered to stably overexpress either RET fusions demonstrated transformed phenotypes. The fusions were constitutively active, as shown by endogenous phosphorylation of the kinase domain, and drove activation of downstream signaling as shown by increased phosphorylation of ERK and AKT. Cells transformed by RET-fusions were exquisitely sensitive to treatment with RET inhibitors. Interestingly, a PDX model of RET-amplified TNBC was sensitive to treatment with a PIK3CA inhibitor. An index case of ER+/PR-/HER2+, metastatic breast cancer that had radiographic evidence of disease progression while on trastuzumab, pertuzumab, and anastrozole was found to have a NCOA4-RET fusion by CGP. Subsequent treatment with with cabozantinib plus anastrozole led a rapid clinical and radiographic response.

Conclusions: CGP can identify recurrent RET rearrangements in breast cancer that act as primary oncogenic drivers and can be therapeutically targeted. RET alterations may also play a role in acquired resistance to HER2-targeted therapies, suggesting a role for combined RET and HER2-targeted therapy in this setting. Our data demonstrate that RET alterations can be identified by clinical-grade CGP and are promising candidates as therapeutic targets in selected breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-03

Title: GPNMB activates EGFR to overcome Mek-inhibition: Implications for the development of rational targeted therapy combinations in triple negative breast cancer

Rose A, Annis M, Perkins D and Siegel P. McGill University, Faculty of Medicine, Montreal, QC, Canada and Goodman Cancer Research Center, Montreal, QC, Canada.

Body: Background:

TNBC is an aggressive subtype that constitutes ~15% of all BC. Currently there are no targeted therapies available for patients with. As such, there is much interest in developing targeted therapies for this disease.

Recently we identified GPNMB as a transmembrane protein that promotes breast tumor growth and metastasis. CDX-011 is an antibody drug conjugate that targets GPNMB, and has recently shown promising clinical activity in patients with GPNMB+TNBC. In subset analyses of the EMERGE trial, patients with high GPNMB expressing TNBC had a median OS of 10 vs. 5.5 months for CDX011 versus chemotherapy, respectively. Response rates to CDX011 correlated with degree of GPNMB expression. These findings support the hypothesis that TNBC with high GPNMB will respond better to CDX011. As such, we sought to identify therapies with intrinsic activity against TNBC that would also induce GPNMB expression, in order to synergize with CDX-011. We have recently shown that MAPK pathway inhibition induces GPNMB expression in melanoma. Therefore we sought to determine whether Mek inhibition (Mek-i) induced GPNMB in TNBC and the targeted therapies could synergize with CDX-011.

Results:

We interrogated the TCGA breast datasets to determine whether the MAPK pathway is more frequently altered in TNBC. Indeed, this pathway is altered in 94% of Basal compared to 60% LumA, 83% LumB, 73% Her2 subtypes. Furthermore, we show that a Mek-activation transcriptional signature is significantly higher in basal compared to Her2 or Luminal subtypes. Indeed, we show that Fra, a downstream target of activated Erk, is most highly expressed in BC cells of the basal subtype.

We used immunoblot and FACS analysis to assess GPNMB expression in response to Mek-i; trametinib and cobimetinib markedly induced GPNMB protein expression in 12 of the 14 TNBC cell lines tested. Moreover, we show that in the TCGA dataset, low expression of the Mek-activity signature correlates with higher GPNMB, specifically within the basal subtype, thus providing clinical corroboration for our *in vitro* observations. We go on to show that Mek-i mediated GPNMB up-regulation is regulated by TFE3.

We find that EGFR is upregulated in response to Mek inhibition in several TNBC cells. GPNMB heterodimerizes with EGFR in immunoprecipitation experiments. Using siRNA to knockdown GPNMB or ectopic GPNMB overexpression, we found that GPNMB is both necessary and sufficient for enhanced EGFR activation in response to Mek-i in TNBC.

Finally, we used CRISPR-CAS9 to delete genomic GPNMB from murine lung-metastatic TNBC cell lines. 533LM2 grow in syngeneic Balb/c mice. Here we show that GPNMB is required for tumor growth and metastasis *in vivo*. Mek-i slows tumor growth; but the combination of GPNMB deletion with Mek-i led to tumor regression, and significantly impaired tumor growth and metastasis relative to all other groups.

Conclusions:

Together our data show that the MAPK pathway is hyperactivated in TNBC; inhibiting this pathway impairs tumor growth, but enhances GPNMB, which facilitates mammary tumor growth and metastasis in the setting of Mek-i. These data provide rationale for combined targeting of GPNMB and the MAPK pathway in TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-04

Title: WNT4 mediates endocrine response and resistance in invasive lobular carcinoma cells

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Body: Invasive lobular carcinoma (ILC) is a breast cancer subtype affecting ~30,000 U.S. women annually. Over 90% of ILC are estrogen receptor (ER)-positive; however, endocrine therapy may have poorer efficacy in a subset of ILC patients versus invasive ductal carcinoma (IDC) patients. This prompted us to assess global ER activity in ILC cell lines MDA MB 134VI (MM134) and SUM44PE (44PE) to identify novel mediators of ER signaling. These analyses identified the Wnt ligand WNT4 as an ILC-specific ER target gene, with an ILC-specific ER binding site (ERBS) at the WNT4 locus. Considering the critical role of WNT4 in normal mammary gland expansion, we hypothesize that ILC cells utilize WNT4 signaling to drive endocrine response and resistance. We assessed whether WNT4 is necessary for ILC cell growth using siRNA. WNT4 knockdown completely blocked estrogen-induced growth in ILC cells but not IDC cells. In parallel, the WNT4 ERBS was only occupied in ILC cells in response to estrogen, but progesterone-induced WNT4 in IDC was not associated with this ERBS. This suggests that, via the ILC-specific WNT4 ERBS, ILC cells drive estrogen-regulated proliferation by hijacking a developmental Wnt pathway. Wnt pathways typically activate β -catenin; however, we observed β -catenin dysfunction in ILC cells and that WNT4 cannot activate β -catenin. Thus, WNT4 signals in ILC cells via a novel non-canonical pathway.

Using long-term estrogen-deprived (LTED) variants of MM134 and 44PE (4 and 2 lines, respectively), we assessed WNT4 in ILC endocrine resistance. WNT4 is over-expressed, but uncoupled from ER, in all MM134-LTED. Conversely, WNT4 is reduced in 44PE-LTED but remains ER-regulated; ER occupies the WNT4 ERBS only in 44PE-LTED cells and not MM134-LTED. Using siRNA, MM134-LTED (high WNT4) are growth-inhibited by WNT4 knockdown, while 44PE-LTED (low WNT4) are insensitive. However, WNT4 knockdown sensitizes 44PE-LTED to endocrine therapy. Taken together, uncoupling and upregulating WNT4 or WNT4/ER cross-talk may represent convergent endocrine resistance mechanisms in ILC. Further characterization of ILC-LTED cells demonstrated WNT4 expression is driven by activated NF κ B signaling in MM134-LTED, and implicated the pluripotency factor Oct4 in regulating WNT4 in 44PE-LTED cells. In both parental ILC cells and ILC-LTED cells, WNT4 leads to suppression of CDKN1A/p21, which is critical for ILC cell proliferation; CDKN1A knockdown partially reverses the effects of WNT4 knockdown. Clinical observations suggest that ER regulates unique pathways in ILC. We identified WNT4 as a downstream effector of endocrine signaling in ILC, with critical roles in both estrogen-induced growth and endocrine resistance. WNT4 signaling may represent a novel target to modulate endocrine response specifically for ILC patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-05

Title: CDK8 inhibition improves the efficacy of ER- and HER2-targeted drugs in breast cancer

McDermott MS S, Chumanovich A, Liang J, Chen M, Altilla S, Hennes C, Roninson IB B and Broude EV V. University of South Carolina, Columbia, SC.

Body: Over 70% of breast cancer patients are estrogen receptor (ER) positive and 25% of patients over-express HER2 making these patients susceptible to therapeutic intervention with ER- and HER2-targeted therapies, respectively. However, intrinsic and acquired resistance to targeted therapies is a significant clinical issue and new therapeutic approaches aimed to preventing and overcoming resistance are urgently needed. We have previously shown that high expression of CDK8, a transcription regulating kinase, is associated with shorter relapse free survival in both ER and HER2 positive breast cancer. We have found that CDK8 inhibition by a selective small molecule inhibitor (Senexin B), by shRNA knockdown or by CRISPR/CAS9 knockout, strongly inhibits estrogen signaling in ER-positive breast cancer cells. Senexin B produces a synergistic growth inhibitory effect with an antiestrogen fulvestrant in all the tested ER-positive breast cancer cell lines *in vitro* and in MCF7 xenograft model *in vivo*. Senexin B treatment also inhibited invasive growth of MCF7 xenografts. CDK8 inhibition suppressed the emergence of estrogen independence upon long-term estrogen deprivation. A highly synergistic growth inhibitory effect occurred when Senexin B was combined with an anti-HER2 monoclonal antibody (a biosimilar of trastuzumab) or with the HER2/EGFR small molecule inhibitor lapatinib. These synergistic effects were observed in all HER2 positive breast cancer cell lines tested including those that exhibit innate and acquired resistance to HER2 targeting therapy. Furthermore, combining lapatinib with Senexin B completely abrogated the emergence of acquired lapatinib resistance. Taken together these results suggest that CDK8 inhibition, when combined with either ER- or HER2-targeted therapies, offers a rational approach to improving the efficacy of targeted drugs in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-06

Title: Inhibition of HDAC6 as targeted therapy for breast cancers

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Body: Due to their unique biology, the homeostasis of cancer cells presents different requirements from non-transformed cells. Targeted therapies that interfere with these requirements have been successfully used as highly selective and low toxic anticancer strategies. Recently, we have identified and validated that viability of inflammatory breast cancers (IBC) depends on histone deacetylase 6 (HDAC6) function. Thus, HDAC6 inhibitors, which are currently being tested in advanced clinical trials for other tumor types, represent a novel targeted therapeutic option for these patients.

We reasoned that additional breast cancers, other than IBCs, may present the same dependency and that identification of patient populations that can benefit from HDAC6 targeted therapy would be necessary in order to rapidly transition this finding into the clinic. By using system biology strategies to interrogate the regulatory circuit of breast cancer cells we have found that HDAC6 activity is highly increased in HDAC6-dependent cells, acting as a master regulator. We have also developed an algorithm (HDAC6-score) based on mRNA expression profiling to evaluate the HDAC6 activity of individual tumor samples. Thus, the HDAC6-score works as a biomarker to easily identify cancers with high HDAC6 activity that are likely to depend on HDAC6 function. Using our HDAC6-score algorithm we have analyzed ~3,000 primary breast cancers. Interestingly, we have found that a group of ~20% of breast cancers that is enriched in hormone receptor positive (HR+) and HER2 positive (HER+) tumors presents an HDAC6-score predictive of good response to HDAC6 inhibitors. To validate our findings, we correlate the HDAC6-score and the growth inhibitory response to the leading HDAC6 inhibitor, Ricolinostat, in preclinical breast cancer models in vitro and in vivo. Our preclinical studies confirmed the high levels of HDAC6 activity in HR+ and HER2+ breast cancer cells as well as their sensitivity to HDAC6 inhibition.

Based on our data we have partnered with Acetylon Pharmaceuticals to formally evaluate the anticancer activity of Ricolinostat in breast cancer patients and the predictive value of the HDAC6 score in a clinical trial that has been recently open (the design of this trial will be presented).

Clinical considerations: Despite the success of Pan-histone deacetylase inhibitors (HDACis) against cutaneous T-cell lymphoma, these inhibitors suffer from ineffectively low concentrations in solid tumors and cardiac toxicity due to its activity against HDAC1, HDAC2 and HDAC3, hindering their progress in the clinic. More-selective HDAC inhibitors represent a novel and promising class of anticancer drugs with wider therapeutic indexes. The leading HDAC6 inhibitor Ricolinostat, which is in Phase II trials for multiple myeloma and lymphoma, has a 20-fold more potency for HDAC6 inhibition than other class-I/II histone deacetylases. Thus, Ricolinostat can be dosed more frequently with better tolerability than non-selective FDA-approved HDACis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-07

Title: Inhibition of death-associated protein kinase 1 enhances chemotherapy action against triple-negative breast cancer

Zhao D, Zhao J, Mazumadar A, Bollu L, Shepherd J, Ma Y, Zhang Y, Hill JL L, Savage MI I and Brown PH H. University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Triple negative breast cancers (TNBCs) are the most aggressive ER negative breast cancers with limited therapy strategies and poor prognosis. P53 gene is frequently mutated in approximately 80% of TNBCs. To identify novel molecular targets for ER negative breast cancer, particularly the more aggressive TNBC, we conducted a human kinome screen and identified death-associated protein kinase 1 (DAPK1) as one of the kinases that are highly expressed in ER negative breast cancer. Deletion or inhibition of DAPK1 suppresses growth of p53-mutant but not p53-wildtype breast cancer cells. Here we investigate whether DAPK1 inhibition will enhance chemotherapy action against p53-mutant TNBCs.

Experimental design and methods: We performed experiments to test cell growth of p53-mutant TNBCs that were treated with DAPK1 siRNA or DAPK1 inhibitors in combination with different doses of chemotherapy drugs including 5-FU (5-Fluorouracil), doxorubicin, cisplatin, PARP inhibitor (BMN673), paclitaxel, gemcitabine and vinorelbine.

Results: Our results show that DAPK1 inhibitors enhance the growth inhibitory effects of cisplatin and PARP inhibitor in p53-mutant TNBCs. Furthermore, combined DAPK1 inhibition (via siRNA knockdown) with cisplatin synergistically inhibits cell growth of p53-mutant TNBCs.

Conclusion: DAPK1 is a novel, promising target for the treatment of triple-negative p53-mutant breast cancer. Our studies demonstrate that DAPK1 inhibition sensitizes TNBCs to the cytotoxic effects of cisplatin or the PARP inhibitor. We are now conducting studies to determine whether DAPK1 inhibition will sensitize TNBC tumors and patient-derived TNBC xenografts to the effects of cisplatin and PARP inhibition. These studies suggest that the combination of DAPK1 inhibition with drugs that interfere with DNA repair will be useful for the treatment of the most aggressive form of breast cancer, triple-negative breast cancer.

Funding: This study was funded by a Susan G. Komen Promise grant (SAB12-00006 to P.H. Brown), a MD Anderson Knowledge Gap Moonshot grant (to P.H. Brown) and a Breast Cancer Research Foundation grant (BCRF 15101807, 2015–2016 to P.H. Brown).

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-08

Title: Molecular subtype-specific expression of long noncoding RNA regulates proliferation of basal-like breast cancer cells

Boatman SM M, Han Y-J, Zhang J, Zheng Y, Yeh AC C and Olopade OI I. University of Chicago, Chicago, IL.

Body: Background: Basal-like breast cancer (BLBC) disproportionately affects younger women and African American women, displaying aggressive clinical behavior with poor outcomes. BLBC is difficult to treat due to its lack of defined molecular targets, and with current treatment, patients often develop metastatic disease. A growing body of evidence points to long noncoding (lnc) RNAs as mediators of tumor progression, suggesting a new class of targets in cancer therapy. While dynamic changes in lncRNA expression are observed across many types of cancer, our understanding of their role in cancer biology is limited. The aim of this study is to identify lncRNAs differentially expressed in BLBC and to characterize the functional features of candidate lncRNA.

Methods: Breast tumors and normal breast tissues were recruited from the U of Chicago Breast Cancer Tissue Bank under IRB approved protocols. Microarray profiling was done using Human lncRNA Array v3 (Arraystar) containing 30,586 lncRNAs and 26,109 mRNAs. Non-poly(A) RNAs were included with ribo-zero RNA-seq (Illumina HiSeq 4000). Molecular subtype of breast tumors was determined by PAM50 intrinsic classifier. In addition, CpG methylation of lncRNA promoter regions was analyzed with TCGA HumanMethylation450 Array data from 588 breast tissues. We performed *in vitro* studies using antisense oligonucleotide (ASO) knockdown and CRISPR-on overexpression in BLBC cell lines. Phenotypic consequences of perturbations of specific lncRNA expression were assessed using proliferation, apoptosis, cell cycle and homologous recombination assays.

Results: Microarray profiling of breast tissues from 30 African American women revealed a unique lncRNA signature in basal-like tumors compared to non-basal like tumors. Hundreds of lncRNAs were specifically expressed in BLBC, with >100-fold differences in some lncRNAs compared to tumors from other subtypes or normal breast tissues. Through statistical and *in silico* analyses we selected lncRNAs displaying increased expression in basal-like tumors (*vs* non-basal tumors) and BLBC cell lines (*vs* non-basal cell lines) with a trend of higher expression in cells isolated from women of African ancestry for functional studies. RNA-seq of 50 additional breast tissues recapitulated the distinct lncRNA clustering within the basal-like subtype. TCGA analysis of CpG islands in several lncRNA promoter regions revealed hypo-methylation when compared to non-BLBC, signifying subtype specific epigenetic regulation. ASO knockdown of candidate lncRNAs significantly increased apoptosis and decreased proliferation while CRISPR-on overexpression decreased cell sensitivity to doxorubicin treatment. Depletion of specific lncRNAs caused an increased proportion of cells in G2 phase of the cell cycle, indicating that overexpression of particular lncRNAs may contribute to aberrant cell cycle progression. Cis and trans-regulation of gene expression by lncRNAs is currently under investigation through RNA immunoprecipitation and genome-wide gene expression profiling.

Conclusion: We propose that subtype specific lncRNAs function as onco-RNAs specific to BLBC, driving cellular proliferation and increasing resistance to DNA damaging chemotherapies through regulation of the cell cycle.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-09

Title: Synergistic effect of combinatorial treatment with maraviroc and tocilizumab on TNBC tumor growth and metastasis in mouse xenograft model

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Body: Triple negative breast cancer (TNBC) as a metastatic disease is currently incurable. Unfortunately reliable and reproducible methods for testing drugs against metastasis are not available. We have previously developed a robust metastatic model in which mice are pretreated with tumor cell-conditioned media (TCM) from human TNBC cells (MDA-MB-231 and SUM149) for 2 weeks prior to tumor cell inoculation. In this model we found reproducible metastases in lymph nodes (LN) and lungs within 4-5 weeks after orthotopic tumor inoculation [1]. We have discovered that the TNBC cells secrete large amounts of interleukin-6 (IL-6) that “educates” lymphatic endothelial cells (LEC) in the LN and lungs. Stat3, a transcription factor, gets activated and induces the synthesis of CCL5 and VEGF among other factors. CCL5 recruits the tumor cells to the LN and lungs; VEGF helps build blood vessels in the LN to facilitate tumor cell survival; VEGF produced in the lung helps the tumor cells extravasate into the lung. We have confirmed the importance of these factors by showing that inhibitors of these factors significantly inhibit metastasis.

In this report, using Maraviroc (CCR5 inhibitor) and cMR16-1 Ab (murine surrogate of the anti-IL-6R antibody), we investigated the effect of the combination treatment on the tumor growth and metastasis of orthotopic tumor xenografts generated from MDA-MB-231-Luc-D3H2LN cells. 2×10^6 TNBC cells tagged with luciferase were suspended in 100 μ l PBS/Matrigel (1:1) and injected s.c. into female 3- to 4-week-old BALB/c nu/nu athymic mice pretreated with tumor cell-conditioned media (TCM) from TNBC cells for 2 weeks. We administered the Maraviroc (8 mg/kg Maraviroc, orally daily) and cMR16-1 Ab (i.p. 3-days per week) for 5 weeks. Our data show that tumor growth was dramatically inhibited by cMR16-1 Ab. Further, the drug combination of Maraviroc with cMR16-1 Ab caused significant reduction of TNBC tumor growth in mice compared to single agents. In addition, we measured thoracic metastases by adding luciferin to mice and measuring luminescence ex-vivo in the IVIS imager. Significantly, both single treatment of Maraviroc and the combination of Maraviroc with cMR16-1 abrogated the thoracic metastasis compared to control and single treatment of cMR16-1.

These findings implicate IL-6 and CCL5 signaling as a critical event in TNBC tumor growth and metastasis via crosstalk between cancer cells and stromal components. Further, these studies suggest that IL-6 and CCL5 act as key regulators orchestrating TNBC metastatic breast cancer. Therefore, we have provided evidence that supports the hypothesis that functional inhibition of the IL-6 and CCL-5 signaling pathway has the potential to circumvent TNBC growth and metastasis.

[1] E Lee et al. Breast cancer cells condition lymphatic endothelial cells within pre-metastatic niches to promote metastasis. *Nat Commun.* 2014 5:4715.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-10

Title: The role of CBP/FOXM1 in triple negative breast cancer

Ring A, Nguyen C, Lenz H-J, Tripathy D, Lang JE E and Kahn M. University of Southern California, Los Angeles, CA and The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Accurate assessment of prognostic and predictive biomarkers (estrogen receptor, progesterone receptor, HER2) plays a critical role in the clinical management of breast cancer. Triple negative breast cancers (TNBCs) lack the expression of all three targets, and no targetable molecular pathways have been identified to date. Hence, TNBCs are treated with non-targeted, cytotoxic chemotherapeutic agents (e.g. paclitaxel), and are characterized by high rates of drug resistance and metastatic relapse. CREB binding protein (CBP) has been implicated in cell growth and malignant transformation in various cancers. CBP is an important co-activator in the β -catenin driven transcription, including the Wnt signaling pathway, which has been implicated in TNBC biology. The Kahn lab has developed a specific CBP-binding small molecule inhibitor, ICG-001. We hypothesized that CBP-signaling plays an important role in TNBC biology and may provide a novel therapeutic target.

Methods: We used TOP-flash assay to quantify Wnt signaling activity in TNBC. ICG-001 treatment combined with RNA Seq was used to characterize the role of CBP in TNBC cell line models. Co-immunoprecipitation (CoIP), protein and gene expression studies, as well as gene knock-down were used for validation. *In vitro* drug resistant cell line models as well as *in vivo* cell line (n=40 mice) and patient derived xenografts (PDX) in NOD scid gamma mouse models (2 patients, n=40 mice per patient) (treatment groups: control, paclitaxel, ICG-001, paclitaxel+ICG-001, n=5 mice per condition, primary and secondary implantation) were used to establish the effect of CBP inhibition via ICG-001 in TNBC on drug resistance and metastasis. We used the TCGA breast cancer data set to substantiate the experimental results.

Results: We demonstrated that gene expression in TNBC is CBP, but not Wnt signaling dependent, and can be disrupted via ICG-001. RNA Seq analysis of TNBC cells treated with ICG-001 revealed Forkhead box M1 (FOXM1) as a potential downstream regulator. CoIP demonstrated that CBP binding to the FOXM1/ β -catenin transcriptional complex. Treatment with ICG-001 revealed that CBP/FOXM1 binding, but not FOXM1/ β -catenin binding, is critical for FOXM1 expression. The PDX mouse models demonstrated that FOXM1 expression correlates with response to chemotherapy and disease recurrence *in vivo*. Treatment with ICG-001 sensitized FOXM1 high tumors to chemotherapeutic treatment and statistically significantly reduced tumor growth in serial transplantation experiments. Comparison of clinical data with FOXM1 expression in tumor samples from patients indicated that high levels of FOXM1 were associated with disease relapse and poor survival outcomes.

Conclusion: CBP/FOXM1 binding is critical for FOXM1 expression. Targeting CBP/FOXM1 binding via ICG-001 could provide a novel therapeutic strategy in TNBC. The use of clinically annotated tissue microarrays containing a total of 430 breast tissue cores (51 TNBC cases) is currently pending to correlate nuclear protein expression of CBP and FOXM1 with survival outcomes in TNBC. FOXM1 and CBP could potentially be of value as predictive biomarkers in TNBC. These results could provide a clinical-translational rationale for patient stratification based on CBP and FOXM1 expression for clinical trials exploring the therapeutic potential of FOXM1 inhibition via ICG-001 in combination with chemotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-11

Title: Inhibition of Pin1 or CDK-mediated Smad3 phosphorylation reduces triple negative breast cancer cell EMT, migration and invasion

Thomas AL L, Hamdan R, Hong A, Lind H, Oppat K, Rosenthal E, Thomas AJ J and Jeruss JS S. University of Michigan, Ann Arbor, MI and Northwestern University, Chicago, IL.

Body: Introduction: Triple negative breast cancer (TNBC) is an aggressive subtype associated with poor outcomes. Accordingly, there is an urgent need to develop novel and targeted therapeutics for patients with this disease subtype. Cyclins D and E and the corresponding activation of CDK4/2 represent promising therapeutic targets for the treatment of TNBC. CDK4/2 can non-canonically phosphorylate Smad3, a key TGF β signaling intermediate, and this phosphorylation is associated with the promotion of cell migration and EMT in cyclin-overexpressing breast cancers. Additionally, CDK-mediated Smad3 phosphorylation facilitates an interaction between Smad3 and Pin1. Pin1 is a cis-trans isomerase that is also overexpressed in aggressive breast cancers and can enable TNBC cell migration. Based on these findings, we hypothesized that blockade of the CDK-mediated Smad3-Pin1 interaction, either through inhibition of Pin1 or CDK-mediated Smad3 phosphorylation, would abrogate TNBC cell migration and invasion.

Methods: Pin1 expression was knocked-down (KD) in MDA-MB-231, MDA-MB-436, and Hs578T TNBC cells by transfection with Pin1-targeting siRNA (siPin1) or control non-specific siRNA (siNS). KD efficiency was confirmed with immunoblotting. Pin KD/TNBC cell migration and invasion assays were performed on uncoated or Matrigel-coated trans-wells, respectively. Media containing 10% FBS was used as a chemoattractant. Following Pin1 KD, immunoblotting was used to evaluate EMT-associated protein expression. To inhibit CDK-mediated Smad3 phosphorylation, TNBC cells were treated with 600 nM of CDK2 inhibitor (CDK2i) for 72 hours. Immunoblotting was then performed to determine Smad3 phosphorylation and EMT-associated protein expression. Co-immunoprecipitation assays were used to examine the impact of CDK2i treatment on the Smad3-Pin1 interaction. Finally, following CDK2i treatment, assays were performed to determine the ability of TNBC cells to migrate and invade.

Results: KD of Pin1 expression in TNBC cells resulted in a decrease in cell migration and invasion when compared to control cells in all the study cell lines. This corresponded with changes in EMT-associated protein expression, including increased levels of ZO-1 and claudin and decreased β -catenin. CDK2i treatment produced a decrease in Smad3 T179 site non-canonical phosphorylation and inhibited Smad3-Pin1 binding. CDK2i treatment also abrogated TNBC cell migration and invasion, paralleling expression changes in EMT-associated proteins with an increase in claudin and decrease in β -catenin.

Conclusions: Inhibition of the Smad3-Pin1 interaction, through KD of Pin1 expression or CDK2i-mediated blockade of non-canonical Smad3 phosphorylation, reduced TNBC cell EMT-type changes, demonstrated by increased expression of the tight junction proteins ZO-1 and claudin and decreased β -catenin, a key player in the WNT pathway. These findings also correlated to a reduction in TNBC cell migration and invasion. Collectively, these data show that the Smad3-Pin1 interaction, facilitated by CDK-mediated Smad3 phosphorylation, is associated with pro-migratory TGF β signaling. Inhibition of this interaction, with CDK2 inhibitor treatment, may provide an important therapeutic option for TNBC patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-12

Title: Targeting SET to disrupt oncogenic CIP2A feedforward loop shows therapeutic potential in triple negative breast cancer cells

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Body: Triple-negative breast cancer (TNBC) remains a difficult-to-treat cancer with a need for new therapeutic target. The serine/threonine protein phosphatase 2A (PP2A) functions as a tumor suppressor and enhancing PP2A activity by PP2A-activating agents is a promising anti-cancer approach. There are intrinsic inhibitors of PP2A including SET and cancerous inhibitor of protein phosphatase 2A (CIP2A), both of which interact and inhibit PP2A, playing oncogenic roles. Here we studied the tumor promoting effects of SET and CIP2A and discovered a potent linkage between SET and CIP2A-feedforward pathway in TNBC. We further demonstrated the anticancer activity of a novel SET/PP2A protein-protein interaction inhibitor, TD-19. Analyzing TCGA and clinical TNBC cohort, SET and CIP2A overexpressions correlated with worse survival in TNBC patients. Tumors from TNBC patients with higher SET expressions were significantly associated with higher Ki-67 and p-Akt expressions [Table 1]. Consistent with clinical results, TNBC cells with ectopic overexpression of SET and CIP2A showed significantly increased cell proliferation, migration and invasion, and colony-formation. Moreover, TD-19 demonstrated anti-tumor effects through inhibiting SET-PP2A interaction and CIP2A/PP2A/pAkt-mediated pathway *in vitro* and in a TNBC xenograft mouse model. Mechanistically, TD19 downregulated CIP2A mRNA via affecting Elk-1 amount in nucleus thereby decreased the binding of Elk-1 to the CIP2A-promotor. Furthermore, given that ERK-dependent phosphorylation of Elk-1 is essential for Elk-1 shuttling from cytoplasm to nucleus and PP2A controls ERK activation (phosphorylation), we hypothesized a CIP2A-feedforward loop consisting of pERK/pElk-1/CIP2A/PP2A may contribute to the oncogenesis in TNBC. Indeed, with TD19 treatment, SET/PP2A interaction was inhibited, PP2A activity was increased, and expressions of the CIP2A-feedforward loop members were suppressed. This loop was validated by knockdown of PP2A and ectopic expression of Elk-1. In addition, ectopic expression of SET increased pAkt, pERK, pElk-1 and CIP2A expressions, suggesting a positive linkage between SET and CIP2A feedforward signaling. Importantly, combining TD-19 with cisplatin demonstrated enhanced anti-proliferative and apoptotic effects in association with CIP2A downregulation *in vitro*. In summary, we discover a novel oncogenic CIP2A-feedforward loop in TNBC and targeting SET to disrupt oncogenic CIP2A feedforward loop shows therapeutic potential in TNBC.

Table 1

	High SET H score (>180) N=21	Low SET H score (<=180) N=70	P value
Age [median (range)]	54 (36-69)	56 (30-88)	0.185
Primary tumor (T)			0.999
1	6 (28.6)	21 (30.0)	
2	13 (61.9)	43 (61.4)	
3-4	2 (9.5)	6 (8.6)	
Nodal status (N)			0.122
0	8 (38.1)	45 (64.3)	
1	7 (33.3)	12 (17.1)	
2-3	6 (28.6)	13 (18.6)	
Stage			0.498
1	2 (9.5)	14 (20.0)	
2	13 (61.9)	41 (58.6)	

	3	6 (28.6)	15 (21.4)	
Grade				0.783
	1	1 (4.8)	4 (5.7)	
	2	8 (38.1)	21 (30.0)	
	3	12 (57.1)	45 (64.3)	
Tumor necrosis		13 (61.9)	40 (57.1)	0.698
Lymphovascular invasion		6 (30.0)	15 (21.4)	0.549
CIP2A [median (range)]		120 (40-220)	100 (30-300)	0.253
p-Akt [median (range)]		200 (65-300)	160 (20-300)	0.011
Ki-67 [median (range)]		30 (0-87.5)	22.5 (0-85)	0.004

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-13

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-14

Title: Targeting ERR- α regulated lactate metabolism eliminates drug-resistant breast cancer cells

Quayle L, Park S, McDonnell DP P, Ottewill PD D and Holen I. University of Sheffield, Sheffield, South Yorkshire, United Kingdom and Duke University, Durham, NC.

Body: Novel therapeutic strategies to eliminate chemo-resistant tumour cells responsible for the development of secondary lesions are essential in order to prevent breast cancer relapse. We have developed an *in vitro* model system enabling isolation of a putative metastasis-initiating breast cancer cell sub-population with a mitotically quiescent, drug-resistant phenotype. We hypothesise that this population is able to utilise oestrogen-related receptor-alpha (ERR- α)-regulated oxidative lactate metabolism and that inhibition of this critical survival pathway will result in elimination of tumour cells that have survived anti-cancer therapies. Flow cytometric monitoring of Vybrant® DiD retention identified a mitotically quiescent sub-population (~0.05%) in MDA-MB-231 human breast cancer cells grown under high glucose (11mM) conditions. DiD-retaining cells accumulated in the G₂/M phase of the cell cycle and were shown to be non-senescent following cytochemical analysis of β -galactosidase activity. Cytotoxicity assays with doxorubicin (0.40 μ M for 72 hours) demonstrated increased survival in the quiescent fraction (40.76%) compared with the rapidly dividing bulk cell population (1.63%, $P \leq 0.0001$). Isolated drug-resistant cells contained a sub-fraction (~1%) that was able to form new clonal populations following cessation of treatment. Mitotically quiescent cells were cultured under high glucose (11mM) or glucose-depleted conditions with high lactate (22mM) in the presence of ERR- α inhibitors Cpd29 (10 μ M) or XCT790 (10 μ M). Colony formation was completely eliminated in Cpd29- and XCT790-treated samples compared to untreated controls. Drug-resistant sub-clones were isolated from the quiescent population following 72 hours treatment with doxorubicin (0.40 μ M) and were cultured under glucose-depleted conditions with high lactate (22mM) in the presence of ERR- α inhibitors Cpd29 (10 μ M) or XCT790 (10 μ M). In both instances, colony formation was completely prevented. We provide the first evidence that blocking cellular energy production through inhibition of ERR- α regulated oxidative lactate metabolism can eradicate a chemo-resistant, quiescent breast cancer cell population. Our data suggest that ERR- α inhibitors may be used in combination with chemotherapy to eliminate minimal residual disease and reduce breast cancer recurrence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-15

Title: Treatment with a Jumonji demethylase inhibitor JIB-04 sensitizes resistant breast cancer cells to chemotherapeutic drugs in an *in vitro* model of intrinsic resistance

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Body: We have recently developed a usable model of panresistance in triple-negative breast cancer. We have shown that only 0.01% cells survive a metabolic challenge involving lack of glutamine in culture medium of SUM149 triple-negative Inflammatory Breast Cancer cell line. These cells, designated as SUM149-MA for metabolic adaptability, are resistant to chemotherapeutic drugs, and they efficiently metastasize to multiple organs in nude mice. The MA cells can survive a variety of challenges in the metastasis process because of their embryo-like nature and high adaptability. Epigenetic state is a key player in cancer evolution. Therefore, a variety of compounds are being developed that would target epigenome for anticancer therapy. We believe that the identification of potentially effective epigenetic drugs can benefit from their evaluation in a usable system of panresistant cancer cells such as our model system of MA cells. This is particularly true for the compounds that may affect cellular adaptability upon a long treatment rather than affecting cell proliferation or apoptosis in a short period. In this study we evaluated JIB-04, a pan-inhibitor of Jumonji family of histone demethylases, for its ability to affect intrinsic resistance in MA cells. First, we found that MA cells are more resistant to JIB-04 treatment in a comparative evaluation with the parental SUM149 cells line. A treatment with 125-250 nM JIB-04 for 18-20 days killed all parental cells but not all MA cells; at least 20 MA cells survived and formed colonies after a 14-days recovery. Next, we asked whether JIB-04 treatment would affect the intrinsic resistance of MA cells, e.g., sensitize them to treatment with chemotherapeutic drugs. In order to assess the most resistant cells in population, these experiments involved treatment of MA cells with 125 nM JIB-04 for 10 days followed by a recovery in drug-free medium for 7 days; then we treated the cells with 5 nM paclitaxel or 100 nM doxorubicin for 6-8 days followed by a recovery for 1-12 days. We found that JIB-04 pre-treatment sensitized MA cells and the parental SUM149-Luc cell line to both these chemotherapeutic drugs as assessed by a dramatic drop in the number of colonies as compared to those obtained after treatment of control cells with the same chemotherapeutic drugs. To consider how our results may apply in the context of evolving disease in patients who are not taking chemotherapeutic drugs, we asked whether JIB-04 treatment would alter expression of surface molecules on resistant breast cancer cells such that they can be destroyed by immune system. In this regard, we specifically found that JIB-04 treatment increased the expression of PD-L1 in MA cells. Published results from a clinical study suggest that PD-L1 expression on breast cancer cells renders them responsive to various therapies, not just immune checkpoint blockade targeting PD-L1. In conclusion, our results suggest a novel approach for evaluating potential anticancer agents such as JIB-04 that would halt cancer evolution and prevent development of resistance to currently offered therapies.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-16

Title: ZBTB2 is a novel therapeutic target for cisplatin-resistance in metastatic breast cancer

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Body: Background: Zinc finger and BTB/POZ domain containing proteins (ZBTB) belong to a class of DNA-binding proteins that involved in development, differentiation and carcinogenesis. Our earlier findings showed that ZBTB2 expression was upregulated in cisplatin-resistant cells when compared with naïve cells. Increase in ZBTB2 expression was associated with ABCG2 drug transporter in drug resistance. However, the molecular mechanism of ZBTB2 in drug resistance and breast cancer metastasis remain largely unclear.

Methods: A cisplatin-resistant triple-negative breast cancer (TNBC) cell line, MDA-MB-231/cis, was used to examine the involvement of drug transporter genes and the drug resistance pathway. Stem cell related genes were studied by RNA-sequencing. Characterization of ZBTB2 on cell proliferation, invasion and epithelial-mesenchymal transition (EMT) process were performed in ZBTB2 siRNA transfected cells. Stem-like cells properties were validated using aldehyde dehydrogenase (ALDH) activity, tumorsphere formation ability and stem cell markers. Metastatic animal model was used to study the genetic changes during metastasis and associated with the findings in clinical samples.

Results: ZBTB2 has a higher expression in TNBC than other breast cancer subtypes. Also, the ZBTB2 expression is more prominent in primary breast tumor tissues when compared with non-tumor counterparts from TNBC patients. Silencing of ZBTB2 significantly reduced cell proliferation, invasion and sensitized cells to cisplatin through induction of apoptosis. Induction of ZBTB2 has been associated with breast cancer metastasis in mice model, where higher expression was seen in metastatic tumors when compared with primary tumors from tumor xenografts. ABCG2 and some epithelial-mesenchymal transition (EMT) markers (Twist 1, Snai2 and MMP-9) have been increased in metastatic tumors. Further, RNA sequencing analysis showed that ABCG2, IL6ST and FGFR1 were upregulated in MDA-MB-231/cis cells and downregulated in ZBTB2 siRNA transfected cells. Silencing of ZBTB2 reduced ALDH activity and tumorsphere formation ability. Treatment with inhibitor of NFkB (Bay11-7085) further decreased cell proliferation in cisplatin-resistant cells when compared with siZBTB2 alone. Expression of Stat3 and b-catenin were reduced upon knockdown of ZBTB2.

Conclusion: Taken together, silencing of ZBTB2 sensitize cancer cells to cisplatin through regulation of EMT markers and stem-like cell properties in TNBC. These findings suggest a novel molecular pathway targeting ZBTB2/stat3/NFkB signaling to combat drug resistance in metastatic TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-17

Title: Squalene epoxidase is a potential metabolic oncogene by amplification with clinical implications in breast carcinoma: An *in silico* pan-cancer study with *in vitro* evidence

Zoppoli G, Brown D, Caffa I, Garuti A, Piras D, Gallo M, Isnaldi E, Nencioni A and Ballestrero A. IRCCS AOU San Martino IST, Genova, GE, Italy; Institut Jules Bordet, Brussels, Belgium and University of Genova, Genova, GE, Italy.

Body: Background: The SQLE gene, located on chromosome 8q24.13 in humans, encodes squalene epoxidase, a rate limiting enzyme in the pathway of cholesterol synthesis. In the past, sporadic reports have suggested that SQLE expression may be under control of its copy dosage. Nonetheless, SQLE relevance in cancer has never been studied in detail, possibly due to a conceptual bias given by the physical proximity of SQLE to the well-known oncogene MYC.

Aims: In the present analysis, we investigated SQLE copy number (CN) – gene expression (GE) association in large, well annotated multi-cancer datasets, and focused on the clinical and pathological correlations of SQLE in breast cancer (BC). Using lasso penalized multiple regression, we generated a model in order to explain the variance in SQLE expression accounted for by methylation and transcription factor expression across the diversity of TCGA studied neoplasms. Finally, we aimed at assessing which effects, if any, SQLE inhibition would generate in BC *in vitro* models.

Patients/Methods: We downloaded and analyzed the processed data from the TCGA repository and from the METABRIC dataset. We assessed by array-CGH and q-RT-PCR a panel of BC cell lines portraying the diversity of SQLE behavior in that disease, and performed cytotoxicity assays with terbinafine, a SQLE inhibitor, as well as SQLE directed RNA interference experiments and duplication-time tests.

Results: Breast, ovarian, and colorectal cancers exhibited the tightest SQLE CN-GE correlation among 8,783 cases from 22 cancer types, with BC showing the strictest one. SQLE promoter hypermethylation and regulation by transcription factors seemingly play a greater role to control SQLE GE in other cancer types compared with BC.

SQLE was overexpressed in aggressive, locally advanced BC, and its overexpression subtended a worse prognosis independently of classical clinical and pathological variables. In our BC models, we observed that inhibiting SQLE caused cell demise in a copy-dosage dependent fashion, and increased replication time only in SQLE expresser cell lines, but not in those without endogenously transcribed SQLE.

Conclusions: Our *in silico* and *in vitro* results strongly suggest that SQLE may behave as a bona fide metabolic oncogene by amplification, and could potentially be a novel treatment target in BC and other cancer types.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-18

Title: Exercise and triple negative breast cancer: Unravelling the anti-neoplastic molecular factors through novel culture method

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Body: Background

Despite advances to ameliorate breast cancer survival, triple negative breast cancers (TNBCs) attribute to highly disproportionate mortalities due to its aggressiveness and poor therapeutic response (Bao et al., *Cancer Medicine* 2014). Despite the lack of target-specific drugs, chemotherapy is the mainstay treatment, warranting more efficacious measures against TNBCs. Studies have shown that physical activity intervention reduced breast cancer risk between 20-80% (Monnikhof et. al., *Epidemiology* 2007) as well as breast cancer related mortality by 34% (Ibrahim et al., *Med Onc* 2010). However, it is clear that such intervention uptake in the population may not be feasible given the longstanding public health drive to increase physical activity. Therefore it is imperative to identify the molecular factors that might be involved in cancer prevention and therapy with the long term goal of developing a supplement. Due to the recent epidemiological findings on exercise and breast cancer, we wanted to develop a system to comprehensively identify beneficial myokines using a cell culture system.

Methods

For this study we differentiated C2C12 myoblasts into myotubules. Myotubules were contracted in Krebs Ringer Buffer solution with the C-Pace EP Pacer for 8 hours. Buffer was collected and concentrated using Amicon Ultra-0.5 centrifugal filter tubes to produced an exercise/conditioned medium. To explore the effects of exercise on breast cancer cell lines MCF-7 (ER+) and MDA-MB468 (TNBC line) were treated with conditioned medium for 48 hrs. To explore the effects of exercise and chemotherapeutic efficacy MDA-MB468 cells were treated with doxorubicin, conditioned medium or both for 48 hrs. Protein was isolated and processed for immunoblot analysis. Cell cycle markers p21, pRb, cyclin D1, PCNA as well as apoptotic marker cPARP were assessed to reveal the effects treatment.

Results

Treatment of conditioned medium in MCF-7 revealed marked changes in both p21 (92% increase, $p=0.05$) and pRb protein expression (62% decrease, $p<0.05$) as well as a modest reduction in PCNA (24% decrease, $p<0.02$). Conditioned medium treatment in MDA-MB468 cells showed a reduction in cyclin D1 and PCNA expression (40%, $p<0.01$ and 30%, $p<0.02$) as well as an induction of cPARP expression (32%, $p=0.09$). Doxorubicin treatment increased p21 and cPARP protein expression (2.2-fold increase, $p<0.01$ and 80% increase, $p<0.05$ respectively). Treatment of conditioned medium and doxorubicin displayed a synergistic effect with a 3.65-fold increase in p21 ($p<0.01$) and 130% increase in cPARP ($p=0.01$).

Conclusions

We have developed a novel system that may enable, for the first time, mechanistic studies to elucidate the role of skeletal muscle/exercise in breast cancer prevention and chemotherapy. Our data indicates a secreted factor(s) from skeletal muscles that plays a role in anti-proliferative and pro-apoptotic effects, which are the hallmark of exercise's anti-neoplastic properties. Studies are ongoing to further understand, characterize and isolate this factor(s) for therapeutic purposes. Given the limited chemotherapeutic options and heterogeneity in TNBC, our exercise culture system sheds a promising light on novel drug development and chemo-sensitization.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-19

Title: Dual inhibition of the MEK5 and PI3K pathways synergistically reduces proliferation and viability in triple negative breast cancer cells

Wright TD D, Raybuck C, Wendekier K and Cavanaugh JE E. Duquesne University Graduate School of Pharmaceutical Sciences, Pittsburgh, PA.

Body: Aberrations in the MAPK/extracellular signal-regulated kinase (MEK/ERK) and phosphoinositide-3-kinase (PI3K) pathways have been linked to increased proliferation and survival in triple negative breast cancer (TNBC) cells. It has been proposed that these survival characteristics are enhanced through compensatory signaling and crosstalk mechanisms. Promising combinations of MEK and PI3K inhibition have been evaluated in phase I clinical trials for various cancer types. However, these clinical trials have had limited efficacy and have yet to encompass the MEK5/ERK5 pathway, which has been shown to promote cell survival. The goal of this study was to examine the crosstalk between the MEK1/2, MEK5, and PI3K pathways and determine the most promising combination of the MEK1/2, ERK5, and PI3K inhibitors, U0126, XMD8-92, and LY294002, respectively, in a diverse panel of triple negative breast cancer cell lines: BT549, MDA-MB-231, and MDA-MB-468. Our results indicate that dual inhibition of the MEK5 and PI3K pathways significantly reduced proliferation (45.53%) in MDA-MB-231 TNBC cells. Also, the combination of MEK5 and PI3K inhibition was shown to be synergistic. In contrast, inhibition of ERK1/2 alone or in combination with PI3K or ERK5 inhibition yielded mixed responses. Additionally, treatment with LY294004 in MDA-MB-231 (ERK 5 KO) was more potent (IC_{50} = 2.5 μ M) than treatment in the native MDA-MB-231 cell line (IC_{50} = 13.7 μ M). These data suggest that crosstalk between these kinases occurs and dual inhibition of PI3K and ERK5 may be a novel therapeutic approach for treating TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-20

Title: Biological functions of ER β in triple negative breast cancer and its utility as a novel therapeutic drug target

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Body: Background: Triple negative breast cancer (TNBC) accounts for approximately 20% of all breast cancer diagnoses. It is the most aggressive form of breast cancer and clinical management is problematic due to lack of available targeted therapies. We have shown that approximately 30% of all TNBCs express estrogen receptor beta (ER β), a ligand binding transcription factor, and a potential drug target for patients with this form of the disease.

Methods: Using novel ER β -expressing TN cell lines developed in our laboratory, we assessed the impacts of ER β on proliferation, invasion, migration, and alterations in cell cycle progression following estrogen and ER β -specific agonist treatment. We also characterized the ER β transcriptome and cistrome in these models through microarray and ChIP-Seq, respectively. Finally, we determined the tumoral response of cell line xenografts and PDXs treated with 17 β -estradiol.

Results: We found that both estrogen and multiple ER β -specific agonists elicit significant anti-tumor effects in ER β + TNBC cell lines and tumor xenografts. Activation of ER β with estrogen and ER β -specific agonists resulted in inhibition of cell proliferation primarily through a G1/S phase cell cycle arrest. Substantial reductions in cell migration and invasion were also observed following treatment. Microarray studies revealed that ER β differentially regulated the expression of approximately 1000 genes following estrogen treatment. Of these genes, the most striking effects were observed in a family of small secreted cysteine protease inhibitors known as cystatins, which were highly induced following ER β activation. ChIP-Seq and ChIP-PCR identified ER β binding sites in the promoter region of each cystatin and demonstrated ER β -mediated alterations in chromatin marks and recruitment of PolII around these promoters. We found that cystatins directly interact with TGF β receptor 2 (TGF β R2) and block downstream TGF β ligand-mediated activation of the canonical signaling pathway. Depletion of cystatins from conditioned media or through siRNA-mediated silencing reduced the ability of ER β to elicit these anti-tumor effects. *In vivo*, estrogen treatment of mice harboring ER β + TNBC cell line xenografts or PDXs resulted in increased tumoral expression and serum levels of cystatins, and suppressed tumor growth.

Conclusions: Our data demonstrated that estrogen and ER β -specific agonists elicit anti-cancer effects in ER β + TNBC, both *in vitro* and *in vivo*. These effects are partially mediated by cystatins which can interact with, and inhibit, canonical TGF β signaling, a pathway known to drive TNBC progression. Given the lack of targeted therapies for TNBC patients, the present data suggests that estrogen or ER β -specific agonists offer a novel approach to manage this subset of patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-07-21

Title: Cytotoxic potential of the RG7388 MDM2-p53 binding antagonist and the GSK2830371 WIP1 inhibitor on MX-1 and MCF-7 human breast cancer cells

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Body: Background

The tumor suppressor p53 is a central hub in molecular signaling pathways that control the integrity of the human genome. The p53 protein functions as a transcription factor and increases the expression of many cellular genes which contribute to activation of cell cycle arrest, apoptosis and DNA repair. *MDM2* is another important p53 target gene, and the MDM2 protein is capable of binding directly to p53 and directing it for degradation through the ubiquitin-dependent proteolytic pathway. Inhibition of MDM2 stabilizes p53 and MDM2 inhibitors are being explored clinically as therapies. Stabilization alone may not be enough to increase the activity of p53, and posttranslational modification of p53 by phosphorylation has been proposed to be an important contributory mechanism by which p53 becomes functionally active. Therefore maintaining the phosphorylated status of p53 in tumor cells may help to enhance its growth inhibitory and pro-apoptotic role. Wild type p53 – induced phosphatase (Wip1) is a serine – threonine phosphatase which dephosphorylates central players in the DNA damage response, including p53 and may be an additional target to enhance p53-dependent treatments. Therefore this work was focused on the effect of MDM2 (RG7388) and Wip1 (GSK2830371) inhibitors on MX-1 and MCF breast carcinoma cell lines. These two cell lines were recorded to have wild type *TP53* status as well as high expression of Wip1.

Trial design

RG7388 and GSK2830371 were tested for growth inhibition on MX-1 and MCF-7 breast cancer cell lines using the sulforhodamine B (SRB) assay. The results were further confirmed and mechanism explored by western blotting using extracted protein from drug treated cell lines. Contradictory evidence regarding the *TP53* mutation status of the MX-1 cell line was clarified by direct sequencing of MX-1 DNA.

Results

The MCF-7 cells responded to both RG7388 and GSK2830371 with GI_{50} value of 0.034 μ M and 2.92 μ M respectively. The MX-1 cells did not respond to either drug. The results of western blotting showed there was no expression of p53 in the MX-1 cell line. Failure to respond to RG7388 and also no expression of p53 in western blotting made us suspicious about the *TP53* status of the MX-1 cells. The direct sequencing results confirmed that there was a 5bp deletion in exon 4 of the *TP53* gene of the MX-1 cells. The c.154_158delCAATG mutation creates a stop codon at the 54th aminoacid position and results in a truncated p53 protein (p.Gln52Valfs*3).

Conclusion

RG7388 and GSK2830371 showed cytotoxic effects on MCF-7 cells, whereas both RG7388 and GSK2830371 had no effect on the MX-1 cell line due to the truncated p53 and loss of p53 function. In conclusion, the potency of both drugs depends on the *TP53* mutation status and they are likely to be mediated via p53-dependent growth inhibition and apoptosis. Further studies are needed to evaluate the combination effect of both drugs on *TP53* wild type cell lines.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-08-01

Title: TruRisk® based next-generation sequencing in *BRCA1/2*-negative breast and ovarian cancer families reveal high mutation prevalence in additional risk genes

Keupp K, Richters L, Bülow L, Kröber S, Ernst C, Blümcke B, Versmold B, Waha A, Driesen J, Baasner A, Altmüller J, Thiele H, Nuernberg P, Wappenschmidt B, Neidhardt G, Rhiem K, Schmutzler R, Hahnen E and Hauke J. Center for Familial Breast and Ovarian Cancer, Center for Integrated Oncology (CIO), Medical Faculty, University Hospital Cologne, Cologne, Cologne, Germany and Cologne Center for Genomics, University of Cologne, Cologne, Germany; Institute of Human Genetics; University of Cologne, Cologne, Germany.

Body: Background: 24% of familial breast cancer (BC) and/or ovarian cancer (OC) cases analyzed within the framework of the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC) are due to pathogenic *BRCA1/2* mutations. However, the mutation prevalence of non-*BRCA1/2* genes associated with familial BC and/or BC/OC is largely unknown. **Methods:** Here, we present the first NGS data generated using the GC-HBOC-designed TruRisk® gene panel. In this study a cohort of 2028 *BRCA1/2* and *CHEK2* c.1100delC negative index cases was analyzed which comprises consecutive patients from BC families and BC/OC families complying the inclusion criteria of the GC-HBOC. Sequencing was performed on MiSeq, NextSeq, or HiSeq devices (Illumina) using customized SureSelect XT enrichment (Agilent). Data analysis was carried out using the SeqPilot software (version 4.2.2), SophiaDDM (Version 3.5.0.12-p5.0.0) as well as an in house bioinformatics pipeline (Cologne Center for Genomics, *varpipe_v2.X*). The analysis of copy number variations (CNV) based on NGS-data is currently in process and not yet included in the present mutation prevalence. **Results:** By focusing on 22 BC/OC associated genes (*ATM, BARD1, BRIP1, CDH1, CHEK2, FAM175A, FANCM, MLH1, MRE11A, MSH2, MSH6, NBN, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, RINT1, STK11, TP53, XRCC2*), we identified 71 different deleterious variants in 104 unrelated mutation carriers derived from 2028 BC and BC/OC families (8%). Interestingly, we identified a high prevalence of *ATM* mutations (n=29, 1.4%) in the familial cases. Additionally mutations in *PALB2* (n=27), *NBN* (n=9), *CHEK2* (n=14), *BARD1* (n=9), *BRIP1* (n=10), *RAD51C* (n=11) were frequently observed and we confirmed *FANCM* (n=17) as a novel BC predisposing gene. No mutations in *MLH1, MRE11A, PTEN, RAD51D, STK11* and *XRCC2* were identified in our collective. **Conclusions:** Due to the unexpectedly high mutation prevalence in familial cases, our study highlights the importance of these genes to be included in BC/OC routine diagnostics. In contrast we found low occurrence or absence of mutations for a subset of our gene selection which requires further investigation to optimize the gene panel for diagnostic purposes. Nevertheless this approach confirms the TruRisk® gene panel as a reliable tool for this comprehensive analysis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-08-02

Title: Multi-gene panel testing for hereditary cancer predisposition in unsolved high risk breast and ovarian cancer patients

Crawford BB B, Adams SB B, Sittler T, Van den Akker J, Chan SB B, Leitner O, Ryan LN N, Gil E and Van 't Veer LJ J. UCSF, San Francisco, CA; Color Genomics, Burlingame, CA and Skypax, LLC, Chapel Hill, NC.

Body: Background

Among women with an elevated risk of hereditary breast and ovarian cancer who previously tested negative for pathogenic mutations in *BRCA1* and *BRCA2*, a subset remain at increased risk of having hereditary breast, ovarian or other cancers, and should be offered multi-gene panel testing. We tested three groups of women who were enrolled in the UCSF Cancer Genetics and Prevention Program: (i) 97 women with a personal history of bilateral breast cancer, (ii) 104 women with a personal history of breast cancer and a first-degree or second-degree relative with ovarian cancer, and (iii) 99 women with a personal history of ovarian, fallopian tube, or primary peritoneal cancer. All women previously tested negative for pathogenic *BRCA1* and *BRCA2* mutations by either limited or comprehensive testing.

Methods

We performed comprehensive next-generation sequencing using a panel of 19 genes developed by Color Genomics (a CLIA-certified laboratory) covering *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *EPCAM*, *MLH1*, *MSH2*, *MSH6*, *NBN*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, *STK11*, and *TP53*.

Results

Across the groups tested, 9% had pathogenic mutations in one or more of the genes analyzed (8% in genes other than *BRCA1* and *BRCA2*). Among these women, Ashkenazi Jewish and Hispanic women had elevated mutation rates compared to those of other ethnicities. In addition, we identified two women with pathogenic mutations in two cancer susceptibility genes, which has significant implications for family testing. These results demonstrate the importance of genetic testing of genes other than *BRCA1* and *BRCA2*.

Conclusions

Among women with an elevated risk of hereditary breast and ovarian cancer who have previously tested negative for *BRCA1* and *BRCA2* mutations, we propose that women with characteristics of any of the three groups above be considered for subsequent multi-gene panel testing. Additionally, ethnicity and the possibility of multiple mutations may be indications for additional testing in these women and in family members of carriers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-08-03

Title: Factors associated with genetic testing in a cohort of breast cancer survivors

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Body: Background: Approximately 35% of individuals with breast cancer meet guidelines for Germline genetic testing based on young age of onset, triple-negative subtype, and positive family history. While the national guidelines expanded opportunities for genetic testing, it is unclear how consistently these guidelines are being followed. It is also unclear which factors influence the decision to receive genetic testing or learn the results of testing. We present the results of a large multi-institution survey examining factors associated with the receipt of genetic testing, adherence to recommended guidelines for genetic testing and accessing results, in addition to evaluating correlates from the medical record compared to self-report.

Methods:

The Greater Plains Collaborative (GPC) Clinical Data Research Network (CDRN) Breast Cancer Group conducted a comprehensive multi-site (7 cancer care delivery sites across the Midwest) survey, *Share Thoughts on Breast Cancer Study*. Inclusion criteria were women >18 years, with microscopically-confirmed ductal carcinoma in situ or invasive (but not metastatic) breast cancer, diagnosed January 2013 to May 2014, and with no prior cancer other than breast cancer. Questionnaire data were linked to tumor registry data for those who consented to access their medical record.

Results:

1235/1987 surveys were completed giving an overall response rate of 61.4%. Signed informed consent to utilize medical records was obtained for 852/1235 (69%). The median age of survivors at diagnosis was 59 years, 90.4% were white, 45.2% had a 4-year college degree or more, and 58.9% had private insurance. 486/1235 (39%) underwent genetic testing with 53 planning to in the future. 8% (39) reported having a deleterious mutation with 7% not knowing the results of their genetic testing. Among younger women with no family history of breast cancer, 74.8% of those under age 50 and 89.3% of those under age 45 reported receiving genetic testing. Of younger women with >1 relative with breast cancer, 86.7% (age <50 years) and 87.5% (age <45 years) reported receiving testing. Of those < 60 years with triple negative tumors, 75.0% reported getting genetic testing. Self-reported factors that correlated with genetic testing include younger age, higher family income (54% of those with household income >\$100,000 were tested vs. 27% of those with household income <\$20,000), higher education level (49% of those with 4 years of college or more were tested vs. 25% of those with high school or less), having private insurance (53% tested, vs. 26% of those reporting government or no insurance), and having had more relatives with breast cancer.

Conclusion:

Our study demonstrates the feasibility of undertaking a comprehensive survey of breast cancer survivors from across the Midwest. Genetic testing rates of 39%, were consistent with national recommendations. However, there is still an opportunity to identify additional survivors who did not undergo testing within 2 years of their diagnosis, particularly those with lower education and poorly insured. There are ongoing efforts to determine why 7% of the cohort did not report knowing the results of their genetic testing.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-08-04

Title: Trends in age of breast cancer diagnosis for women with pathogenic variants in genes associated with increased breast cancer risk

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Body: Background: The National Comprehensive Cancer Network (NCCN) currently recommends consideration of genetic testing for appropriate, high risk individuals when it will impact medical management of the individual or at-risk family members. Established NCCN testing criteria are based on family history, the presence of multiple primary cancers, and age of diagnosis. For breast cancer, women diagnosed before age 50 are eligible for genetic testing with limited family history; however, these criteria were developed based on high-risk breast cancer genes, such as *BRCA1* and *BRCA2*. The growing use of gene panels has extended testing to include genes associated with a 2- to 4-fold increased risk for breast cancer. Although NCCN guidelines now include medical management recommendations for these genes, it is unclear whether current criteria appropriately identify candidates for testing who have pathogenic variants (PVs) in genes with moderate breast cancer risk. Here, we investigated the age of breast cancer diagnosis in women carrying PVs in genes with high or moderate breast cancer risk.

Methods: Clinical testing was performed for 68,239 women with a personal diagnosis of breast cancer using a 25-gene hereditary cancer panel that includes genes with a high (*BRCA1*, *BRCA2*, *PTEN*, *TP53*) or moderate (*PALB2*, *CHEK2*, *ATM*, *STK11*, *CDH1*, *NBN*, *BARD1*) risk of breast cancer. The majority of women tested met current NCCN criteria for testing based on their personal and/or family cancer history. The proportion of women with a PV who were diagnosed <50 and <60 years of age was evaluated.

Results: Overall, 5,231 women diagnosed with breast cancer were found to carry a PV in a gene with high or moderate breast cancer risk. 70.2% of women with PVs in genes with a high breast cancer risk were diagnosed with breast cancer before age 50, compared to only 55.5% of patients with PVs in genes with moderate breast cancer risk (see Table). However, similar proportions of women with PVs in genes with a high (89.3%) and moderate (80.2%) breast cancer risk were diagnosed before age 60 (see Table).

Gene	Diagnosed <50	Diagnosed <60
Moderate Breast Cancer Risk		
<i>CHEK2</i>	458 (59.3%)	629 (81.5%)
<i>PALB2</i>	337 (54.7%)	507 (82.3%)
<i>ATM</i>	315 (52.4%)	452 (75.2%)
<i>BARD1</i>	68 (51.5%)	111 (84.1%)
<i>NBN</i>	56 (52.3%)	86 (80.4%)
<i>CDH1</i>	24 (58.5%)	33 (80.5%)
<i>STK11</i>	4 (66.7%)	6 (100%)
Total	1262 (55.5%)	1824 (80.2%)
High Breast Cancer Risk		
<i>BRCA1</i>	1086 (76.4%)	1314 (92.4%)
<i>BRCA2</i>	901 (63.0%)	1227 (85.8%)
<i>TP53</i>	61 (83.6%)	68 (93.2%)
<i>PTEN</i>	28 (90.3%)	31 (100%)
Total	2076 (70.2%)	2640 (89.3%)

Conclusions: Approximately half of the women with a PV in a moderate breast cancer risk gene identified here were diagnosed before age 50. This likely overestimates the proportion of moderate-risk PV carriers with early onset breast cancers, as current testing criteria are weighted towards diagnoses at young ages. Given that there are now medical management guidelines for patients who carry PVs in most of the moderate-risk breast cancer genes, it is important to consider whether current testing criteria developed for genes with a high breast cancer risk effectively identify women with PVs in moderate-risk genes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-08-05

Title: The yield of germline genetic testing in breast cancer patients diagnosed prior to age 50

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Body: Young breast cancer (BC) patients are more likely to carry a mutation in a cancer predisposition gene than women diagnosed later in life. Historically young BC patients were eligible for BRCA1/2 genetic testing, but now with the advent of larger panels, more extensive germline genetic testing is available. Little is known about the yield of panel testing in young BC patients. The goal of this study is to report on the testing outcomes of referrals to a single institution cancer genetics program for women who had a diagnosis of BC at or prior to age 50.

METHODS: Cases were identified from the cancer registry of a single institution. Women with invasive breast cancer or DCIS were included if their diagnosis was at or before age 50. Charts were reviewed to abstract data on age of diagnosis, type of testing offered, and results of genetic testing, as well as insurance status and family history of cancer.

RESULTS: A total of 386 young BC patients were referred for genetic counseling in 2011-2015. Of those, 287 (74%) attended a genetic counseling appointment. Many of the women not attending had previously had genetic testing with an outside provider; their test results were included in the table below. Of the 287 attending a genetics consultation, most were offered genetic testing (87.1%); most of those not offered testing had either already been tested but without genetic counseling, or did not meet current NCCN or their insurer's criteria for testing. Eighteen women were offered a panel but elected to have only BRCA testing due to concerns about variants of unknown significance (VUS). Of those tested, 15.4% had a pathogenic or likely pathogenic mutation identified. Of women who had only BRCA testing, 11.7% were positive and 4.4% had a VUS. Of those who had panel testing, 16.9% were positive and 19.3% had a VUS.

Percentage of women with positive tests by age at diagnosis

Age at Dx	30 or less (n=18)	31-35 (n=39)	36-40 (n=78)	41-45 (n=109)	46-50 (n=108)	Total (n=352)
BRCA1/2	27.8%	18.0%	16.7%	6.4%	6.5%	11.1%
Other genes	11.1%	5.1%	3.8%	4.6%	2.8%	4.3%
Total yield	38.9%	23.1%	20.5%	11.0%	9.3%	15.4%

Other genes with positive panel results included 4 x TP53 mutations, 4 x CHEK2, 2 x PALB2 and 1x each of RAD51C, RAD51D, STK11, NF1, and PTEN. The three patients who tested positive for a hereditary cancer predisposition syndrome (STK11, NF1, and PTEN positive) had clinical features of their respective syndromes. The STK11 patient was previously known to have Peutz-Jeghers syndrome, while the NF1 and PTEN patients had subtler features of Neurofibromatosis type 1 and PTEN-Hamartoma Tumor Syndrome (Cowden Syndrome) respectively.

CONCLUSIONS: The yield of germline genetic testing in BC patients increases with younger age of diagnosis. Panel testing increases the yield of testing above that of BRCA1/2 alone, and enabled the formal diagnosis of a few individuals with hereditary cancer syndromes who did not have classic features of their syndromes. The VUS rate for panel testing remains significant, and some women elect to have BRCA1/2 testing only due to personal preference because of VUS rates. These data describing the yield of testing in BC patients diagnosed at a young age may be useful for genetic counseling of this patient population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-08-06

Title: Screening, management, cancer diagnoses, and outcomes of women with germline BRCA mutations in Israel: The Noga Clinic experience

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Body: Background: Women with germline BRCA (gBRCA) mutations in the United States are typically diagnosed at the time of cancer diagnosis and most often undergo bilateral mastectomy. Israel's population of 8 million includes over 3 million Jews of Ashkenazi ancestry, of whom 2.5% would be expected to have gBRCA based on population studies. Cross-ancestral and -racial marriages in Israel continue to expand the population at risk for gBRCA mutations. Gene typing for any woman in Israel can be obtained without cost through either the national health care system or genetic screening studies. The Noga Clinic (NC) in Jerusalem was started in September 2007 for women with documented gBRCA mutations at risk for breast and/or ovarian cancer to counsel them on risk-reducing (rr) surgical interventions and provide rigorous screening to diagnose cancer at its earliest stages. Screening is performed in compliance with NCCN and international recommendations. This report presents information from that unique population and clinical experience.

Methods: Clinical records of women with at least one screening visit at NC through December 2015 were retrospectively reviewed. Women with a documented gBRCA mutation with or without a clinical history of breast/ovarian cancer were eligible for assessment every (q) 6 months (m): bilateral breast exam by an experienced breast surgeon (q6m), serum CA-125 (q6m), trans-vaginal ultrasound (q6m), bilateral mammography (q12m) and bilateral breast ultrasound (q12m) alternating with bilateral contrast enhanced MRI (q12m) beginning at age 25. Women are offered rr bilateral mastectomy (BMast); rr salpingo-oophorectomy is recommended by the age of 40. Cancer diagnoses and rr surgeries were recorded.

Results: 611 women have undergone at least one screening assessment at the NC, of whom 44 had a prior cancer diagnosis. Age at the time of initial gene testing ranged from 20-87y (median 38). Of those with recorded mutation data, 272 women (57%) had mutations in gBRCA1, 205 (43%) in BRCA2, and 1 in both. Mutation specifics will be presented in detail. For the 567 women without a prior cancer diagnosis, median age at first screening visit was 37y (range 21-87) and total follow up was 2,141 person-years (per person range 1-9y, median 3.3). Only 21 women (4%) elected rrBMast at a median age of 42y (range 26-60); none developed breast cancer. In the remaining individuals, 31 breast cancers were diagnosed (58% gBRCA1, 42% gBRCA2) from the initiation of screening at a median age of 51y (range 28-88); the majority were DCIS and diagnosed at a median screening follow up of 2.8y. No participant was treated with anti-endocrine chemoprevention. There have been no breast cancer deaths. 5 women were diagnosed with ovarian cancer (all gBRCA1) of whom 1 died of the disease. Methods of cancer detection will be presented.

Conclusion: This is the largest reported data set of women with gBRCA mutations without a prior breast or ovarian cancer diagnosis screened and followed over time. In this highly selected predominantly Jewish population in Israel, the great majority of women chose not to undergo rrBMast and have excellent outcomes when participating in regular and rigorous screening. Further follow-up is ongoing.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-08-07

Title: ATM mutations contribution to hereditary breast-pancreatic cancer

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Body: BACKGROUND: Germline mutations in *PALB2*, *BRCA2* and *STK11* are well established as increasing risk of both breast and pancreatic cancer. More recently, *ATM* and *BRCA1* mutations have also been associated with risk, but literature is limited. We investigated the prevalence of pathogenic mutations and likely pathogenic variants (“mutations”) in *BRCA1/2*, *PALB2*, *STK11* and *ATM*, comparing mutation occurrence in individuals with diagnoses of breast cancer alone to those with both breast and pancreatic cancer primaries. Prevalence of *CDKN2A* (*p16*) mutations was also evaluated in the breast-pancreatic cohort because of its contribution to hereditary pancreatic cancer.

METHODS: Clinical histories and test results were reviewed for patients undergoing multi-gene panel testing at one clinical laboratory between April 2012 and June 2015. The study population was limited to women with breast cancer only (n=27,573) and women with both breast and pancreatic cancer (n=97) without other primaries. Patients underwent comprehensive analysis of 5-49 genes, depending on the panel ordered. Demographic and clinical information was provided by clinicians on test requisition forms and pedigrees/clinic notes if provided. Gene-specific mutation frequencies were compared between women with breast cancer only and women with breast and pancreatic cancer using Fisher's exact test.

RESULTS: Mutations were identified in *BRCA1*, *BRCA2*, *PALB2* or *ATM* in 13 of the 97 breast - pancreatic cancer probands (13.4%) and 1,255 of the 27,573 breast cancer probands (4.6%). Gene-specific mutation frequencies and statistical comparisons may be found in Table 1. *ATM* mutations were significantly more likely to be identified in women with breast and pancreatic cancer compared to breast cancer alone (Table 1). Interestingly, no *CDKN2A* or *STK11* mutations were identified in the breast plus pancreatic cohort, although this may have been limited by the small number of individuals tested for this gene. Of those 13 women with breast and pancreatic cancers who had identified mutations, 11 (85%) had diagnoses of breast cancer over age 50.

Table 1. Mutation Frequencies

Gene	Mutation Frequency n/N (%)		p	OR (95% PI)
	Breast & Pancreatic	Breast Only		
ATM	6/89 (6.74%)	209/17,570 (1.19%)	0.00076	6.00(2.12,13.85)
BRCA1	2/90 (2.22 %)	429/26,336 (1.63%)	0.66	1.37(0.16,5.14)
BRCA2	4/90 (4.44%)	442/26,336 (1.68%)	0.066	2.72 (0.72,7.28)
CDKN2A	0/54 (0.00%)	13/3,965 (0.33%)	1	0.00 (0, 24.70)
PALB2	1/89	1.12% 175/17,570 (1%)	0.59	1.13(0.03, 6.54)
STK11	0/86	0.00% 0/16,931 (0.00)	1	Inf (0, Inf)

CONCLUSION: This exploratory study substantiates the association of deleterious germline *ATM* mutations with predisposition to both breast and pancreatic cancers. These results also suggest that mutations in *ATM* may account for a larger portion of inherited breast and pancreatic cancer kindreds than mutations in other well-described genes such as *BRCA2*, *PALB2* and *STK11*. A personal history of breast and pancreatic cancer may warrant the expansion of current NCCN testing criteria as a single indicator for germline testing, and that pancreatic screening consortia (CAPS) consider inclusion of *ATM* mutations in screening recommendations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-08-08

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-08-09

Title: Social media's impact on patient utilization of high-risk clinics for genetic counseling and testing services

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Body: Background: The availability and variety of cancer genetic testing services have grown tremendously in past years; testing services now offer panels that analyze more than 75 cancer susceptibility genes with results available in just weeks. Engaging the high-risk population in conversations regarding the importance of hereditary cancer screening is a prerequisite to initiating genetic testing and appropriate cancer surveillance. It has been shown that population awareness of cancer genetic testing has increased, but few studies have explored factors that contribute to actual uptake of these services.

Methods: A 36-question research survey was adapted from Cycle 3 of the 2014 Health Information National Trends Survey by NCI and distributed online to patients who received genetic counseling at the Ruth Paul Hereditary Cancer Program at the George Washington University Medical Faculty Associates. All patients who presented to the clinic, including those with and without cancer diagnoses, were invited to complete the survey. The survey was divided into four sections: (1) how the patient usually seeks health information, (2) how the patient has used media and the internet to understand cancer genetics, (3) how often the patient visits health professionals, and (4) why the patient pursued testing at the high-risk clinic.

Results: Forty-five out of 68 consented individuals completed the online survey. Most patients searched the internet regarding genetic testing in the past (64% vs. 36% who did not) but felt that they learned the most about cancer genetic testing through their health care provider and their family members/friends (49% and 35%, respectively). Though most patients access social networking sites (78%), only 4% shared information and 11% received information regarding cancer genetic testing through one of these sites. Most patients (65%) felt that information obtained from social media regarding cancer genetic testing had no impact on their decision to make an appointment. One-third of respondents (33%) felt that Angelina Jolie's decision to have prophylactic surgery for her known mutation encouraged them to make an appointment while 53% felt it had no impact on their decision. Most patients received a referral from their healthcare providers (91%) to have genetic testing, though 48% of those patients had to ask their doctor about genetic testing before receiving the referral.

Conclusions: Many patients use social media and internet resources for education regarding cancer genetic testing. However, most individuals sought genetic counseling services in our Hereditary Cancer Program after discussion with their health care providers. Half of those patients were only referred after raising the topic with their provider. While online resources can raise awareness and educate about cancer genetic counseling, improving uptake and utilization of these critical resources will require education of health care providers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-08-10

Title: Highly accurate hereditary risk assessment tool for low-income breast cancer patients

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Body: Introduction: Genetic testing is known to improve outcomes in high-risk women by finding cancers in the earliest most treatable stage or through prophylactic measures. However, these life-saving services may not be available to low-income women due to lack of insurance or access to genetic providers. To address this need, a collaboration between the Hereditary Cancer Clinic at Vanderbilt-Ingram Cancer Center (VICC) and the Robert E. Hardy Cancer Clinic at Nashville General Hospital at Meharry Medical College (MMC) was established in 2015 to systematically screen all MMC breast cancer patients for hereditary traits and refer them for genetic counseling (1). We hypothesized that high risk women could be accurately identified using this clinic based screening tool.

Methods: MMC clinic staff screened breast cancer patients using a 10-item Family Cancer Risk Assessment tool (RISK) that has been designed for use in a busy clinic environment (2). We tested the accuracy of the RISK by comparing the results to a 3-generation pedigree and the current NCCN guidelines for referral of patients to genetic services (3). The project was approved by the IRBs at each institution and study data were collected and managed using RedCap electronic data capture tools hosted at Vanderbilt University (4). Summary statistics and Chi-square for significance were performed.

Results: 73 breast cancer patients completed the RISK during their clinic visits and 41 (56%) had a high-risk score of 6 or more. All 41 patients have been referred for genetic counseling, with 18 (44%) women having completed a pedigree interview over the phone. 11 of these 18 patients (61%) were African-American; 5 (27.7%) were Caucasian; and one each (5% each) were of Asian and Hispanic ancestry. 9/18 were diagnosed \leq 50 years (Mean entire group =50 yrs; range 36 -57). 5/18 (27.7%) had triple negative markers on pathology and 2/18 were ER+/PR+/Her2+, and the remainder had ER+/PR+/HER2- cancers. Among the 18 patients with full pedigrees, 17 (95%) patients met current NCCN guidelines based on pedigree analysis. The one outlier had a revised RISK score based on updated information obtained during the pedigree interview. Genetic testing was offered to 10 patients seen in VICC clinic and 1 declined testing. The other 8 patients either failed (n=4) or are awaiting an appointment (n=4). No deleterious mutations were seen in those tested. 4 VUSs (BRCA2, NBN, SMARCA4, and RAD51D) were found in 3 of the 9 tested patients. No significant differences were found in race, age or type of tumor.

Conclusion: Point of care risk assessment using the Family Cancer Risk Assessment screening tool is highly accurate for identifying patients at high genetic risk for hereditary breast cancer. While the tool was completed using pen and paper, it could easily be computerized for ease of administration and calculation of risk scores. This approach benefits the busy oncologist in identifying and referring appropriate patients for genetic testing.

1. Funds awarded by GreaterGood.org. 2. Joseph G. et al. 2012 *Public Health Genomics*. 3. National Comprehensive Cancer Network: Genetic/High-risk Breast_Ovarian (Version 2.2016). 4. Harris et al. 2009. *J Biomed Inform*.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-08-11

Title: Contribution of germline mutations in cancer predisposition genes to tumor etiology in women diagnosed with invasive breast cancer before 40 years

Ellsworth RE E, Rummel SK K and Shriver CD D. Chan Soon-Shiong Institute of Molecular Medicine at Windber, Windber, PA and Murtha Cancer Center, Washington, DC.

Body: Background: Although breast cancer in young women (YW) accounts for <10% of diagnoses annually, tumors in young patients have more aggressive characteristics and higher mortality rates. The cost of breast cancer, including treatment costs, physical and psychosocial effects, and lost productivity, is higher in YW than older patients. Improved understanding of etiology of breast cancer in YW is critical to developing effective prevention strategies.

Methods: All patients diagnosed before 40 years were identified. Family history was classified as average (No first or second degree relatives with breast or ovarian cancer or 1 second degree relative with breast cancer diagnosed >50 years), moderate (1 first degree relative with breast cancer, 2 first or second degree relatives with breast cancer diagnosed >50 years or 1 first or second degree relative with ovarian cancer) or strong (≥ 1 first or second degree relative with bilateral breast cancer, breast and ovarian cancer or male breast cancer, ≥ 2 first or second degree relatives with breast cancer before age 50, breast and ovarian cancer in different relatives, ovarian cancer at any age or >3 first or second degree relatives with breast cancer at any age). Genomic DNA was isolated from blood samples and targeted sequencing was performed using the TruSight Cancer panel (Illumina). Pathogenic mutations were identified using VariantStudio.

Results: Seven percent (132/1950) of patients enrolled in the CBCP were diagnosed <40 years. Of these, 7% had a strong family history). TruSight sequencing was completed for 63 women for whom genomic DNA was available: five patients had pathogenic BRCA2 mutations (1813dupA, 5849del4, 999del5, Q2491X, Y3098X), all ER+ tumors, and seven patients had BRCA1 mutations (187delAG, 448insA, 943ins10, E84X, Q1313X, E1535X) all in triple negative breast cancers (TNBC). A pathogenic CHEK2 I157T was detected in an African American woman with TNBC. Variants of unknown significance were also detected in APC, ATM, BRCA1, BRCA2, CHEK2, CDH1, ERCC4, FANCA and PMS2 and heterozygote mutations detected in autosomal recessive genes BLM and RECQL4.

Discussion: Pathogenic mutations were found in 21% of young women with breast cancer with an additional 22% harboring potentially pathogenic mutations. BRCA1 mutations were associated with triple negative breast tumors in individuals with moderate to strong family history and BRCA2 mutations were associated with ER+ tumors in young women without strong family histories. These data demonstrate that although genetic predisposition may account for 21-43% of tumors, >50% of tumors in young women are not attributable to genetic causes, and identification of those non-genetic factors is critical to reduce the burden of breast cancer in this population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-09-01

Title: BRCAsearch - results of population-based screening of BRCA1 and BRCA2 germline mutations in incident breast cancer in South Sweden

Loman N, Borg Å, Henriksson K, Kristoffersson U, Kvist A, Silfverberg B, Törngren T, Brandberg Y and Nilsson M. Lund University, Lund, Sweden; Skåne University Hospital, Lund University, Lund, Sweden; Laboratory Medicine Region Skåne, Lund, Sweden and Karolinska Institutet, Stockholm, Sweden.

Body: Increasing evidence supports the benefit of identifying the BRCA1 and BRCA2 germline mutation status in early breast cancer. Germline mutations in these two genes are treatment predictive for the benefit of risk reducing surgical interventions of the contralateral breast and the ovaries and fallopian tubes, in early breast cancer (Metcalf 2014; Domchek 2010). Furthermore, they may possibly also be treatment predictive for the effect of certain types of medical interventions (Tutt SABCS 2014). In order to provide the patient with the opportunity to take BRCA test results into account already when planning primary treatment, BRCA testing and cancer genetic counseling needs to be initiated and performed early during the diagnostic and treatment related process in newly diagnosed breast cancer cases. Special attention needs to be taken to the fact that the test results may affect not only the patient, but also her or his relatives. We have observed that the clinical efficacy of BRCA-screening criteria may be surprisingly low, leaving a substantial number of BRCA-carriers undetected in spite of intense contacts with health care providers (Unpublished results).

BRCAsearch (ClinicalTrials.gov Identifier: NCT02557776) is a population based study including new invasive breast cancer cases at three hospitals in the South of Sweden. Patients are invited to perform BRCA1 and BRCA2 germline mutation analysis at time of primary treatment after written study information and written genetic counseling, irrespective of family history, age or clinical phenotype. The primary end-points of the study include the rate of BRCA mutations in newly diagnosed breast cancer in South Sweden, the fraction of patients that accept inclusion in the study, and the rate of mutation positive patients that do not fulfill clinical criteria for BRCA mutation screening. Secondary end-points include the perception among patient of the intervention and testing procedures, the types of questions the patients present during the process and psychosocial outcome among carriers and non-carriers.

In May 2016 about 400 patients have been included in the study, whereof 333 have been analyzed for BRCA germline mutations. The accrual target of the study is 500 patients, which will be reached during the fall of 2016. At the meeting, the BRCA1 and BRCA2 mutation rates will be presented, as well as the acceptance rate of the study among patients approached, and the fraction of mutation positive patients that do not fulfill current BRCA1 and BRCA2 mutation screening criteria, and whose positive mutation status therefore would have been missed if clinical selection criteria had been applied.

The results of the study will be used to define selection criteria for BRCA-mutation screening in new breast cancer cases, and the design of screening procedures with an appropriate clinical effectiveness.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-09-02

Title: Unhealthy lifestyle patterns are prevalent in unaffected BRCA mutation carriers & are associated with increased oxidative stress and telomere length alterations

McGarrigle SA A, Guinan EM M, Hussey J, O'Sullivan J, Boyle T, Hanhauser Y, Al-azawi D, Kennedy MJ, Gallagher DJ J and Connolly EM M. Trinity College Dublin, Dublin, Ireland and St. James's Hospital, Dublin, Ireland.

Body: The lifetime-risk of breast-cancer is greatly increased in women carrying a deleterious mutation in the *BRCA1* or *BRCA2* genes. Recently, there has been increased penetrance of *BRCA1* and *BRCA2* mutations which may be due to lifestyle influences. There is a need to identify approaches to reduce the penetrance of BRCA 1/2 mutations. Understanding how modifiable lifestyle-factors affect cancer-risk in BRCA-mutation carriers may have implications for risk-reduction in this group. At the molecular level, oxidative-stress and telomere dysfunction are early events in cancer development and these processes may be considered surrogate markers of cancer-risk. It has been reported that BRCA-mutation carriers are more susceptible to these pro-carcinogenic processes than non-carriers.

The aim of this pilot study was to objectively measure lifestyle factors in unaffected BRCA-mutation carriers and to assess the impact of these lifestyle-factors on oxidative-stress profiles and telomere length.

Participants (n=75) were recruited from breast-cancer family-risk clinics and cancer-genetics clinics. Body-composition (BMI, waist-circumference), metabolic profiles and physical-activity (triaxial accelerometry) were measured for each participant. Serum levels of the oxidative-stress markers 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-DG) and 4-hydroxynonenal (4-HNE) were measured in a subset of participants (n=30) by ELISA. Telomere length was measured in a subset of participants (n=30) by quantitative PCR (qPCR).

Participants demonstrated poor adherence to physical-activity guidelines with 94% not reaching physical-activity levels recommended by the American College of Sports Medicine. The majority of participants were overweight (39%) or obese (32%) with 73% exhibiting abdominal obesity. 21% of participants had the metabolic syndrome (MetS) at the time of study enrolment with the majority of participants (80%) presenting with at least one feature of the MetS. Circulating levels of 8-oxo-DG did not appear to be affected by body composition or MetS status, however, serum levels of the lipid peroxidation marker 4-HNE were significantly higher in participants with the MetS ($p < 0.0001$). Correlation of serum 4-HNE levels with individual features of the MetS and related parameters revealed significant direct correlations with waist circumference ($p = 0.02$), number of features of MetS ($p = 0.0007$), insulin ($p = 0.02$) insulin resistance score (HOMA-IR) ($p = 0.01$), HBA1c ($p = 0.006$), glucose ($p = 0.048$) and triglycerides ($p < 0.0001$). Age-adjusted telomere length was not influenced by anthropometric measurements or MetS status in this group. Moderate physical activity levels were inversely associated with age-adjusted telomere length; particularly, among post-menopausal participants ($p = 0.009$).

This work has provided compelling evidence that in this cohort of BRCA-mutation carriers, unhealthy lifestyle-patterns are prevalent. In addition, these results suggest that the potential may exist to modify pro-carcinogenic processes in this cohort by modifying physical activity levels and targeting the metabolic syndrome and its component features lifestyle interventions and/or medication.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-09-03

Title: Low-level gonosomal mosaicism of a de novo *BRCA1* gene mutation – The origin of a constitutional mutation in a breast cancer family

Bülow L, Keupp K, Richters L, Pohl E, Wappenschmidt B, Zarghooni V, Reichstein-Gnielinski S, Maringa M, Giesecke J, Rhiem K, Hahnen E and Schmutzler R. Center for Familial Breast and Ovarian Cancer, Center for Integrated Oncology (CIO) and Center for Molecular Medicine Cologne (CMMC), Medical Faculty, University of Cologne and University Hospital Cologne, Cologne, Germany.

Body: Mosaicisms arise when specific cells within a developing organism mutate to result in two or more cell populations with distinct genotypes. In cases of gonosomal mosaicism a genetic variation is present in both somatic and germline cells. Here, we describe a large Turkish breast cancer family with four affected individuals. In the Index patient (II-1; age of onset 45 years), a heterozygous deleterious frameshift mutation, c.1310dupA, p.His437Glnfs*2 in *BRCA1* was identified using the TruRisk® gene panel designed by the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC). Predictive genetic testing showed heterozygous carrier status in the daughter (III-1). The mutation was also analyzed in peripheral blood of the affected mother (I-1; age of onset 45 years) of the index patient by Sanger Sequencing. Interestingly, Sanger sequence did demonstrate the presence of remarkable small peaks presenting the frameshift mutation similar to a mosaic pattern. A second and third independent blood draw within a time frame of four month was tested and a mosaic signal of approximately 10 % was reproducibly detected. In order to exclude a potential allelic drop out, independent sequencing experiments via next generation sequencing (NGS; TruRisk® gene panel) were performed. Again, the mutation was present with an allele read frequency of 12 %. No other pathogenic mutations were detected in any of the other tested breast cancer susceptibility genes. To further examine and underscore the presence of gonosomal mosaicism different tissues should be analyzed. As no tumor material or surrounding normal breast tissue was available primary skin fibroblasts were isolated from skin biopsy. Sequencing of cultured primary fibroblasts demonstrated the absence of the familial *BRCA1* mutation. With NGS-based CNV analysis as well as MLPA analysis we excluded aberrant copy numbers of *BRCA1* in blood and fibroblasts. In conclusion, our data provide striking evidence for a *BRCA1* mosaicism, which is not detectable in all body cells. Due to the inheritance of the *BRCA1* mutation to the next generations (II-1 and III-1), we assume the presence of a gonosomal mosaicism in the affected mother (I-1). Additionally, the history of breast cancer onset in the family indicates that the *BRCA1* mosaic mutation carrier do not necessarily have a milder phenotype compared to full heterozygotes. In this context our results implicate the importance of using highly sensitive sequencing platforms in routine diagnostics to ensure the detection of disease causing low-level mosaic mutations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-09-04

Title: Beyond *CHEK2* in breast cancer: Search for additional moderately penetrant risk gene variants by analyzing the oligogenic disease course in *CHEK2* mutation carriers

Pohl E, Richters L, Hauke J, Ernst C, Kröber S, Niederacher D, Arnold N, Ramser J, Groß E, Gehrig A, Schmidt G, Dutrannoy V, Kast K, Hahnen E and Schmutzler R. Center for Familial Breast and Ovarian Cancer, Center for Integrated Oncology (CIO) and Center for Molecular Medicine Cologne (CMMC), Medical Faculty, University of Cologne and University Hospital Cologne, Cologne, Germany; University Hospital Düsseldorf, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany; University Hospital of Schleswig-Holstein, Campus Kiel, Christian-Albrechts University Kiel, Kiel, Germany; Klinikum rechts der Isar, Technical University Munich, Munich, Germany; Institute of Human Genetics, University Würzburg, Würzburg, Germany; Institute of Human Genetics, Hannover Medical School, Germany; Institute of Human Genetics, Campus Virchow Klinikum, Charite Berlin, Berlin, Germany and University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany.

Body: In one third of all cases, breast cancer (BC) shows a familial aggregation, suggesting a genetic predisposition. Besides *BRCA1/2*, several highly or moderately penetrant risk genes could be identified, including *CHEK2* (cell-cycle checkpoint kinase 2) which controls DNA repair upon DNA damage, cell cycle arrest, and apoptosis. The *CHEK2* germline mutation c.1100delC is a European founder mutation causing a frameshift, premature protein truncation (p.T367Mfs*15), and loss of protein function. It has been shown that *CHEK2* c.1100delC carriers are predisposed to estrogen receptor positive (ER+) breast tumors. As the absolute risk of BC and the probability of developing bilateral BC in *CHEK2* c.1100delC mutation carriers seems to be higher in women with a BC family history (Cybulski et al., 2011), many previous studies were interested in identifying additional moderately penetrant risk gene variants explaining the *CHEK2* specific phenotype. Although the multiplicative nature of risk effects due to *CHEK2* mutation c.1100delC and additional moderate or low risk variants have been suggested in the past (Byrnes et al., 2008; Sokolenko et al. 2014), additional moderate risk genes in *CHEK2* mutation carriers have not been studied so far. Here we focused on 165 *BRCA1/2* negative BC/OC index cases recruited by the German Consortium for Hereditary Breast- and Ovarian Cancer (GC-HBOC) which were heterozygous for one of the two European founder mutations (c.1100delC or delExon9/10). Samples were analyzed by targeted next generation sequencing using a 48 gene panel including confirmed BC risk genes like *ATM*, *PALB2* or *FANCM* as well as Lynch syndrome causing genes and several putative novel risk genes which have been identified recently or are currently under investigation in national and international studies. We were able to identify a total of 9 patients (9/165; 5.5%) carrying at least one deleterious mutation in addition to the known heterozygous *CHEK2* mutation of which one carried mutations in 2 genes (*FANCL*, p.S144Lfs*6 and *PMS2*, c.250+3_250+6del). Deleterious variants in the remaining patients were identified in *ATM* (p.I1581Nfs*5), *FANCL* (p.S144Lfs*6), *FANCM* (p.Q156*), *GPRC5A* (p.R61Sfs*59, 2 times), *PMS2* (p.D414Rfs*44), *RINT1* (p.Q356*) and *XPC* (p.C670*).

In summary, the results obtained with this selection of genes further supports the assumption that the increased cancer risk for *CHEK2* mutation carriers with a family history of BC/OC is at least in part explainable by an oligogenic disease course.

Preliminary data suggest an association of the presence of additional deleterious mutations and an earlier age of onset in *CHEK2* mutation carriers. For instance the patient carrying two mutations in *FANCL* and *PMS2* in addition to the *CHEK2* c.1100delC variant showed a remarkably low age of onset (31 years). Analysis of missense and potential splice site variants in this cohort are mandatory to further validate these findings. Additionally, we could confirm panel diagnostics as a powerful tool to improve individual risk prediction and clinical care of *CHEK2* mutation carriers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-09-05

Title: Clinical outcome of patients with advanced triple negative breast cancer with germline and somatic variants in homologous recombination gene

Stjepanovic N, Kim RH H, Wilson M, Mandilaras V, Berman H, Amir E, Cescon D, Elser C, Randall Armel S, McCuaig J, Volenik A, Demsky R, Chow H, Misyura M, Wang L, Oza AM M, Kamel-Reid S, Stockley T and Bedard PL L. Princess Margaret Cancer Centre, Toronto, ON, Canada.

Body: Background: Variants in homologous recombination (HR) genes other than *BRCA1/2* may cause a *BRCA*-like phenotype triple negative breast cancer (TNBC), which includes the sensitivity to platinum and DNA repair inhibitors. Evaluation of HR proficiency may influence the clinical management of TNBC. Our aim was to evaluate germline and somatic HR gene variants in advanced TNBC patients (pts) and clinical outcome.

Methods: Our cohort included advanced TNBC pts unselected for family history or age at diagnosis, enrolled in an institutional molecular screening program (NCT01505400). DNA from matched blood and FFPE tumor samples was assessed using a lab developed next generation sequencing Hereditary Cancer Panel (NGS-HCP) that includes all exons of 52 cancer predisposition genes, with 20 HR genes (Illumina MiSeq/NextSeq, germline coverage 100x, somatic coverage 500x). Medical records were reviewed for clinical outcome, pathology and prior germline *BRCA1/2* testing results. All pts consented for research on banked samples and return of pathogenic germline variants was optional. Log rank test was used to determine time from surgery with curative intent to relapse (TTR) and overall survival from diagnosis to death (OS) differences based on presence of HR variants.

Results: We included 32 pts who consented for return of pathogenic germline variants and had sufficient DNA for NGS-HCP analysis. Median age at diagnosis was 45 years (range 21-80). Initial stages at diagnosis were: I (12.5%), II (62.5%), III (19%) and IV (6%). Germline HR variants were detected in 17 pts (53%) with a median number of variants per patient of 1 (range 0-6). Five pts had likely pathogenic or pathogenic variants in HR genes: *BRCA1* (2), *BRCA2* (1), *FANCC* (1) and *FANCC + BML* (1). Another patient had a *BRCA1* pathogenic variant previously detected by Multiplex Ligation-dependent Probe Amplification but was not detected by NGS-HCP. 26 variants of unknown significance (VUS) were identified in 13 HR genes, including *FANCA* (6), *FANCF* (3) and *BRCA1* (3). Only one patient had a somatic HR variant in *FANCA* not found in the germline. 30 pts (94%) had somatic *TP53* variants. Sporadic somatic *BRCA1/2* variants were not seen. *BRCA1/2* variants present in the tumor were equivalent to those detected in blood of *BRCA1/2* carriers. Median (m) TTR was 17 months (range 1-119) and mOS was 49 months (range 8-123). Presence of likely pathogenic or pathogenic germline variants was not associated with TTR ($p=0.78$) and OS ($p=0.23$). Presence of germline VUS, likely pathogenic or pathogenic variants also did not correlate with TTR ($p=0.72$) and OS ($p=0.47$)

Conclusions: In our cohort of pts with advanced TNBC, 12% had germline pathogenic variants in *BRCA1/2*, similar to the previously reported rate in early stage TNBC pts. Prevalence of likely pathogenic or pathogenic variants in non-*BRCA* HR genes was 6%. The presence of germline variants in HR genes was not associated with clinical outcome, however, the number of patients included was small and we had limited power to detect survival differences.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-01

Title: Investigation of HBOC germline mutations in women diagnosed with breast cancer in Trinidad and Tobago

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Body: Background: Trinidad and Tobago (T&T) is a southern Caribbean island with a population of approx. 1.3 million. According to WHO/PAHO, T&T has the 2nd highest breast cancer mortality rate in the region, and breast cancer continues to lead in incidence and mortality in cancers among female citizens. Notably, a large proportion of breast cancer cases in T&T appear to occur at a young age; with nearly 36% of breast cancers diagnosed under the age of 50. It is known that a younger age at diagnosis can be associated with Hereditary Breast and Ovarian Cancer syndrome (HBOC), characterized by germline mutations in tumor suppressor genes such as *BRCA*, *PTEN* and *TP53*. Yet, the prevalence of HBOC mutations remains unknown in T&T, as local health services for genetic counseling and testing in T&T currently do not exist. As such, our study aimed to determine the prevalence rate of HBOC mutations among women with breast cancer in T&T who met NCCN criteria for evaluation for HBOC syndrome; thus investigating whether there is a need to include genetic testing and counseling in local oncology management in T&T.

Methods: At the National Radiotherapy Center, the main oncology unit in T&T, female breast cancer patients, who met the NCCN criteria for further genetic counseling/testing, were recruited either through doctor referrals or chart reviews. We conducted interviews inquiring about their personal breast cancer diagnosis, risk factors for breast cancer, as well as any relevant family history. This was followed by the collection of saliva samples using Oragene kits, which were then analyzed by Color Genomics Inc. for 30 genes associated with hereditary cancers; including 19 HBOC associated genes such as BRCA1, BRCA2, TP53 and PTEN. Finalized results were returned to patients by genetic counselors from Color Genomics.

Results: 165 patients who met NCCN guidelines for HBOC counseling/testing were approached, of whom 150 agreed to participate. Due to funding and resource limitations, 60 samples were collected thus far; ages ranged from 19-56. The majority of women met NCCN criteria based on age of personal breast cancer diagnosis (40%), and/or family history of breast and/or ovarian cancer (60%). Preliminary results show that of 49 samples, 10 patients tested positive for deleterious HBOC germline mutations: 7 - BRCA1, 2 - BRCA2 and 1 - PTEN; thus far giving a prevalence rate of 20%.

Discussion: Therefore, our results found a strikingly high HBOC germline mutation prevalence rate of 20% among a cohort of female breast cancer patients meeting NCCN criteria in T&T. As we continue to finalize the incoming data on these patients, we hope to draw associations between their genetic status and their respective tumor markers, family history, and personal medical history. However, these initial results demonstrate the need to include genetic counseling and testing in the local oncology management in T&T, as the identification of HBOC mutations can influence treatment options, as well as help identify family members who are at high risk for cancer predisposition. Ultimately, this integration could help alleviate the country's high incidence and mortality rates with respect to breast cancer, and save the public healthcare system significant financial resources.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-02

Title: Increasing participation in research - breast cancer (Inspire-BrC)

Swain S, Robinson BN N, Newman AF F, Tefera E, Herbolzheimer P, Nunes R, Gallagher C, Randolph-Jackson P, Omogbehin A, Dilawari A, Pohlmann P, Mohebtash M, Ottaviano Y, Mohapatra A, Lynce F and Mete M. MedStar Health Research Institute, Washington, DC; MedStar Washington Hospital Institute, Washington Cancer Institute, Washington, DC; Lombardi Comprehensive Cancer Center, MedStar Georgetown University Hospital, Washington, DC; MedStar Union Memorial Hospital, Baltimore, MD; MedStar Franklin Square Medical Center, Baltimore, MD and Georgetown University Medical Center, Washington, DC.

Body: Background: Increasing Black patients' participation in cancer clinical trials is important because of the population's lower survival rate. Accrual for Blacks is the lowest of all groups at 0.5-1.5%. Our study aims to increase trial participation rates among Black breast cancer patients by testing the effectiveness of a culturally tailored video intervention on the decision to participate in a clinical trial.

Methods: We hypothesized that the intervention would increase clinical trial enrollment by 6 percentage points compared to our 2012 enrollment baseline of 6% (22/384). Self-identified Black patients with invasive breast cancer at 5 MedStar Hospitals watched a 15' video about clinical trials, targeting six cultural and attitudinal barriers to participation. The Attitudes and Intention to Enroll in therapeutic clinical Trials (AIET) pre-/post-/follow-up tests with 31 items was used to determine the impact of the video on three domains: actual trial enrollment; likely participation in trials; and attitudes toward trials. The pre-test was conducted at baseline; post-test immediately after video; and follow-up 7-21 days after the intervention. Participants were followed for 6 months to assess trial enrollment status. Descriptive statistics were used to describe study subjects with respect to basic characteristics; means and standard deviations for continuous variables; and frequencies and percentages for categorical variables. Repeated measures analysis of variance was used to examine whether the changes in attitudinal barriers were statistically significant over time. The primary outcome measure was the proportion of Black breast cancer patients who signed consent and/or enrolled in a therapeutic clinical trial.

Results: From Mar/2014 to Sept/2015, 279 patients were approached to join INSPIRE-BrC prior to discussion about therapeutic clinical trials; 52 declined participation. 208 signed consent and 200 completed it. Average age was 59 yrs (SD=12), 75% were stage I-III; 29% were married; 85% had 1 or more children; 29% attended some college or technical school; 53% had private insurance, 31% Medicare, 16% Medicaid; and 53% had a household income <\$40,000/yr. A total of 41 INSPIRE-BrC participants (20.5%) signed consent and 29 (14.5%) enrolled onto a therapeutic study (one-sided $p=0.027$ vs $H_0: P=0.06$). Pre-video, 52% of patients expressed that it was likely they would participate in a hypothetical therapeutic clinical trial; immediately post-video, 67% ($p<0.001$) and 7-21 days after the intervention, 64% ($p=0.003$). Among 31 AIET items, 25 (81%) showed statistically significant and positive change after the intervention. Specifically, trust in the doctor increased and, suspicion in trials decreased ($p<0.001$). Further, patient views on fairness for treatment of poor people and Blacks became significantly more positive ($p<0.001$).

Conclusion: Study findings show that the video is a promising tool for rapid dissemination of a theory-driven, evidence-based model to enhance clinical trial accrual among Black cancer patients. The video also has the potential to positively change attitudes about clinical trial participation.

The study was supported by the Breast Cancer Research Foundation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-03

Title: Socioeconomic disparities in needle biopsy prior to breast cancer surgery across physician referral networks

Killelea BK K, Herrin J, Soulos PR R, Pollack CE E, Forman HP P, Yu J, Xu X, Tannenbaum S, Wang S and Gross CP P. Cancer Outcomes, Public Policy and Effectiveness Research Center, Yale School of Medicine, New Haven, CT; Yale School of Medicine, New Haven, CT; Johns Hopkins School of Medicine, Baltimore, MD; Yale School of Medicine, New Haven, CT; Yale School of Medicine, New Haven, CT; Section of Cardiology, Yale University School of Medicine, New Haven, Ct and Health Research and Educational Trust, Chicago, IL.

Body: Introduction

Although needle biopsy (NB) is recommended prior to breast cancer surgery, the use of NB has been shown to vary according to patient socioeconomic status (SES), operating surgeon, and geographic region. We hypothesized that surgeons who work in the same peer referral network (defined by patient sharing) might have similar practice patterns with regard to NB, and that the magnitude of SES disparities might vary across networks. We therefore examined: 1) SES disparities in the receipt of NB, 2) variation in NB across networks, and 3) whether the association between SES and NB varied across networks.

Methods

We used the SEER database and 5% Medicare sample to examine all patients with a new diagnosis of breast cancer from 2004 through 2006. We used Medicare claims to construct peer groups of physicians based on patient-sharing ties. Patients were assigned to peer groups based on the surgeon who performed their definitive surgery. We defined a patient as having low SES if she was in the lowest quintile of area-level income. We used hierarchical generalized linear models (HGLM) to assess the association between low SES and receipt of NB, including random effects for the surgeon, peer group, and Hospital Referral Region (HRR). We then allowed the low SES effect to vary across peer groups in order to determine whether the association between SES and NB varied across groups.

Results

In the full sample of 14,552 patients, 9,498 (65%) received needle biopsy. In bivariable analysis, patients in the lowest income quintile were less likely to receive NB compared to all other patients (59% vs 67%, $p < .001$). The majority of the variance (59%) in NB use was at the patient level, 22% was at the surgeon level, and 13.7% at the peer group level. The use of NB varied substantially across peer groups, with a median of 69% (interquartile range [51%, 84%]). Even after accounting for physician, peer group, and HRR variation, patients in the lowest stratum of SES were significantly less likely to have received NB compared to all other patient (OR = 0.88; $p = .04$). Finally, we found that the association between SES and NB varied significantly across referral networks ($P < 0.05$).

Conclusions

Patients with low SES are significantly less likely to receive NB prior to breast cancer surgery, and moreover the magnitude of this SES-related disparity varies significantly according to which referral networks are providing care. Future policies to increase NB rates and standardize care for all breast cancer patients may consider the implications of how care for patients with low SES varies across surgical provider networks.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-04

Title: Disparities in human epidermal growth factor receptor 2 testing completion: A population-based retrospective cohort study, 2010-2013

Schroeder MC C, Neuner JM M, Xia C and Thomas A. University of Iowa, Iowa City, IA; Medical College of Wisconsin, Milwaukee, WI and ProMedica Hematology/Oncology Associates, Sylvania, OH.

Body: Introduction: Human epidermal growth factor receptor 2 (HER2) is an established biomarker predictive for response to HER2-targeted therapies. Testing breast cancer specimens for HER2 protein overexpression or gene amplification is the standard of care. Earlier work showed inconsistent delivery of HER2 testing to some patient groups. We sought to understand current imbalances in HER2 testing. Methods: Our retrospective cohort analysis of Surveillance, Epidemiology and End Results Program data included women diagnosed with microscopically confirmed, first primary malignant breast cancer from 2010-2013. Women were categorized by race, stage, age, and whether they received HER2 testing. Those with unknown information in any of these variables were excluded. Descriptive analyses and multivariate logistic regression assessed the effect of these factors on the likelihood of not receiving HER2 testing. Results: The full cohort included 182,032 women, of which 3,551 (2.0%) did not undergo testing for HER2 (Table 1). The portion of HER2 untested tumors was higher for African American women (2.3%) and other non-white women (2.2%), women over age 70 (2.3%) and women with Stage I (2.3%) or Stage IV disease (5.0%). Within the African American cohort similar patterns were seen: age ≥ 70 (2.8%), Stage I (2.8%) and Stage IV (5.4%). The portion of women with untested tumors decreased from 2010 to 2013: 2.6% to 1.6% ($p < 0.001$) for the full cohort, and 2.9% to 1.9% ($p < 0.001$) for African Americans. On multivariate analysis, odds of not undergoing testing were highest for African American women (OR=1.27, $p < 0.001$), women of age ≥ 70 (OR=1.26, $p < 0.001$), and women with Stage IV disease (OR=4.07, $p < 0.001$) (Table 2). Odds of not undergoing HER2 testing decreased over time (OR=0.60 for 2013 vs 2010, $p < 0.001$). Conclusion: Persistent, and significant, disparities in completion of HER2 testing suggest that reasons for not testing extend beyond technical issues. While proportionally small, given the prevalence of breast cancer, addressing these disparities in HER2 testing may offer an opportunity to deliver life-prolonging, often well tolerated, therapy to significant numbers of patients. The improvements in delivery of HER2 testing seen over time are reassuring.

Table 1. Portion of women not having HER2 testing.

		Full Cohort			African Americans		
		N	%	p-value	N	%	p-value
N		3,551	2.0		470	2.3	
Race							
	White	2,708	1.9	<0.001			
	African American	470	2.3		470	2.3	
	Other	373	2.2				
Age at diagnosis							
	<50	708	1.7	<0.001	105	1.9	0.024
	50-59	892	1.9		130	2.3	
	60-69	894	1.9		120	2.4	
	70+	1,057	2.3		115	2.8	
Stage							
	I	2,069	2.3	<0.001	223	2.8	<0.001
	II	762	1.3		111	1.4	
	III	221	1.0		44	1.4	

	IV	449	5.0		92	5.4	
Year of diagnosis							
	2010	1,066	2.6	<0.001	132	2.9	<0.001
	2011	943	2.1		144	2.8	
	2012	801	1.7		93	1.7	
	2013	741	1.6		101	1.9	

Table 2. Multivariate logistic regression for odds of not having HER2 testing.

		OR*	p-value
Race			
	White	ref	
	African American	1.27	<0.001
	Other	1.24	<0.001
Age at diagnosis			
	<50	ref	
	50-59	1.08	0.128
	60-69	1.01	0.795
	70+	1.26	<0.001
Stage			
	I	ref	
	II	1.87	<0.001
	III	0.81	<0.001
	IV	4.07	<0.001
Year of diagnosis			
	2010	ref	
	2011	0.80	<0.001
	2012	0.66	<0.001
	2013	0.60	<0.001

* Odds of group not being tested vs being tested, compared to the reference group and controlling for all other factors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-05

Title: Successful implementation of a novel breast cancer navigation program in Nuevo León, México

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Body: Introduction: Although the overall incidence of breast cancer (BC) is lower in Latin America than in high-income countries, the mortality burden is greater, mainly due to presentation at more advanced stages. Advanced BC presentation has been directly associated to delays by the patient and provider. In Mexico, it has been previously shown that the longest delay occurs within the health system interval, with a median of 5 months. Navigation programs have been proved to be a valuable strategy to assist individuals in overcoming barriers for care across the cancer care continuum. However, its implementation has been limited to date in low-income settings.

Objectives: The aim of this project was to propose a novel BC navigation program to overcome different common barriers for access to accurate diagnosis and early treatment in a limited setting in Nuevo Leon, Mexico. The secondary objectives were to educate and involve medical students by being part of the navigation process and raising women awareness about BC.

Methodology: Through an alliance with the local Ministry of Health, during 18 months, we invited women who had their mammogram done in a referral mammographic center to participate in this Navigation Program. Two weeks after recruitment women were taken by a designated transportation to a specialized BC diagnostic unit, participated in a BC self-awareness educational session (increment in knowledge was measured by pre and post tests), received a clinical breast exam and were provided with their mammography report (interpreted by a breast radiologist). When appropriate, core biopsies were performed and onward referrals to a BC Treatment Center were provided. Along with this process, a Navigator Training Program took place as part of the medical students' 5th year curricula in the ITESM Medical School, within their academic clinical practice (knowledge increment was measured by pre and post tests). This project was supported by Susan G. Komen Foundation.

Results: 1632 low-income uninsured women were navigated and 37 BC were diagnosed. All BC patients started treatment within 3 months from their initial mammogram. 55% were diagnosed at early stages (0-IIA) and 45% at II-B to IV stages (vs. 26% and 74%, respectively, from the overall report from the Ministry of Health in 2014). Patient BC awareness knowledge increased by 22%. 170 students participated in the navigation process, and their knowledge rose by 18% from baseline. The BC Navigation Training Program is now part of the official academic curricula of the ITESM Medical School.

Conclusions: Our Navigation Program was intended to break down medical care barriers to reduce delays and improve quality of care, by guiding Mexican women through the whole continuum of BC diagnosis and early treatment. This successful implementation resulted in shorter health system intervals and BC down staging. Well-designed patient navigation projects should be implemented in low- and middle-income countries to establish their value and feasibility. Due to a lack of educated patient navigators in these regions, health care professionals should be trained to provide navigation guidance through the complex health care systems frequently encountered in underserved settings.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-06

Title: Genetic testing for HBOC among women with a personal diagnosis of breast cancer in patients with Medicaid as compared to patients with private insurance

Baron P, Johnson-Isidore K, Miller L, Brown K, Kidd J, Saam J and Lancaster J. Roper St. Francis Breast Center, Charleston, SC; Myriad Genetic Laboratories, Inc., Salt Lake City, UT; Roper St. Francis Physician Partners, Charleston, SC and Levine Cancer Institute, Charleston, SC.

Body: Introduction: National guidelines recommend that women diagnosed with early-onset breast cancer and/or a strong family history receive *BRCA1/2* testing to guide treatment decisions. Among newly diagnosed patients, a positive test result will often prompt more aggressive surgical treatment to minimize the risk of second primary cancers. Currently, coverage for genetic counseling and testing for Hereditary Breast and Ovarian Cancer (HBOC) under the Medicaid expansion program of the Affordable Care Act has varied by state, where some states require a copayment for this service. Similarly, there is no mandate to cover risk-reducing surgery for patients found to carry a genetic mutation despite research showing cost-effectiveness. This analysis sought to determine whether genetic testing for HBOC among patients with breast cancer is different for those with Medicaid compared to those with private insurance.

Methods: A commercial laboratory database was analyzed for patients with a personal history of breast cancer who underwent testing with a 25-gene hereditary cancer panel from September 2013-February 2016. Patients were eligible for inclusion if they were between ages 18 and 64 at the time of testing and had not undergone previous genetic testing. A total of 17,020 patients with either Medicaid (N=4,313) or one of 5 private payers (N=12,707) were tested during this period. Descriptive statistics, including means for continuous variables and proportions for categorical variables, were calculated. Chi-square tests were used to test associations and differences of positive rates between insurance provider category. Two-tailed p-values are reported, and any p-value less than 0.05 is considered statistically significant.

Results: Medicaid patients had a median age of breast cancer diagnosis of 45 compared to 47 for patient with private insurance. Among women with Medicaid insurance, a higher proportion were of African (13.3% vs 6.4%) and Latin American ancestry (16.4% vs 5.3%). The mutation positive rate among patients with Medicaid was 13.0%, which was statistically higher than patients with private insurance (9.5%) (p<0.001). The positive rate was higher among Medicaid patients of all ancestries suggesting that this discrepancy was not due to ancestry difference among the two testing populations.

Positive rate by ancestry

	Medicaid	Private	Overall
African	80 (13.9%)	72 (8.9%)	152 (11.0%)
Ashkenazi	3 (17.6%)	20 (15.3%)	23 (15.5%)
Asian	26 (13.3%)	40 (7.5%)	66 (9.1%)
Caucasian	201 (12.9%)	171 (9.9%)	918 (10.4%)
Latin American/Caribbean	98 (13.8%)	65 (9.6%)	163 (11.8%)
Native American	7 (13.7%)	9 (7.9%)	16 (9.7%)
Neareast/Mideast	10 (17.9%)	8 (9.3%)	18 (12.7%)
Multiple	49 (12.3%)	77 (9.0%)	126 (10.0%)
None Specified	85 (11.3%)	199 (8.8%)	284 (9.4%)
Total	559 (13.0%)	1207 (9.5%)	1766 (10.4%)

Conclusions: Overall, the positive mutation rate among individuals with Medicaid insurance was higher than those with private

insurance, suggesting the testing requirements applied to this population may be more stringent than those applied to the private insurance population. Consistent genetic testing insurance criteria are necessary for all patients to receive care in line with guidelines following a breast cancer diagnosis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-07

Title: Impact of enrollment in clinical trials on survival of metastatic breast cancer patients

Le Du F, FUJII T, Park M, Liu D, Hsu L, Gonzalez-Angulo A-M and Ueno NT T. The University of Texas MD Anderson Cancer Center, Houston, TX and Eugène Marquis Cancer Center, Rennes, France.

Body: Background: The number of patients worldwide with metastatic breast cancer (MBC) who participate in therapeutic clinical trials has remained so far low. One of the reasons is a lack of opportunity, whereas another is fear of health care providers and patients of poor outcome of the use of new drugs. We therefore investigated whether survival in patients with MBC who participated in first-line therapeutic clinical trials is worse than that in patients who received only standard care and never enrolled in a clinical trial. We hypothesized that first-line therapeutic clinical trials do not negatively affect survival outcome. **Methods:** We reviewed the records of 5501 patients with MBC treated at The University of Texas MD Anderson Cancer Center from January 2000 to December 2010. We extracted a first cohort of 285 patients enrolled in a *therapeutic clinical trial* for the first time for first-line systemic MBC treatment. The second cohort, referred to as the *eligible control population*, consisted of 367 patients without comorbidities who did not participate in a clinical trial at any stage of their disease; these patients lived in Harris County, in which MD Anderson is located, and thus could be potentially eligible for MD Anderson's financial assistance program. To limit confounding factors, we excluded male patients and patients with known brain metastasis from both cohorts. **Results:** The median follow-up duration in our cohort was 7.16 years (95% confidence interval [CI], 6.53-7.64 years). We observed discrepancies in race (minorities were less represented in the clinical trial arm), estrogen receptor (ER) status (more ER-positive than ER-negative patients participated in clinical trials), and metastatic disease site (fewer patients with bone metastasis participated in clinical trials) between the two groups. Overall, we observed no significant differences in progression-free survival (PFS) or overall survival (OS) duration between the treatment arms. Specifically, in the clinical trial cohort, the median PFS duration was 7.00 months (95% CI, 5.72-8.71 months), and the median OS duration was 28.48 months (95% CI, 22.70-34.60 months). In the control cohort, the median PFS duration was 10.02 months (95% CI, 7.13-11.99 months), and the median OS duration was 28.71 months (95% CI, 24.41-31.31 months) ($P = .089$ and $.335$, respectively). **Conclusion:** In MBC patients, enrolling in first-line therapeutic clinical trials does not result in worse survival than does never enrolling in a clinical trial. This study should reassure health care providers in demonstrating that enrollment in a clinical trial does not negatively affect survival in MBC patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-08

Title: Socio-demographic predictors for delay of treatment among a racially diverse, urban breast cancer population

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Body: Background:

Racial and ethnic disparity in breast cancer outcomes is a long-standing problem that continues to worsen. Recent evidence has underscored the importance of timely care, as delays in treatment can lead to decreased survival. We sought to understand predictors of delay to first treatment among a racially diverse cancer population.

Methods:

This is a retrospective study of breast cancer cases diagnosed between the years 2000 to 2014. Data was extracted from the cancer registry at Boston Medical Center (BMC), an urban safety net hospital. Inclusion criteria were any breast cancer diagnosis from 2000-2014, receiving first treatment at BMC, and disease stage 0-III. Cox Proportional Hazards regression analysis was performed to identify predictors of time to first treatment, defined as days from date of diagnosis to date of first treatment with surgery or neo-adjuvant chemotherapy. Covariates included age, sex, race, type of insurance, marital status, US birth, disease stage and year of diagnosis.

Results:

Patients were 99% female and with a mean age of 58 years (range 23-96). Among a sample of 1545 breast cancer cases, 1372 (89%) had surgery as first treatment and 173 (11%) had neoadjuvant chemotherapy. Median days to treatment was 45 days (IQR 27, 71). In the multi-variable adjusted models for both treatment groups, race, and insurance were significantly associated with delay. In the surgical group, Black race, public or no insurance, non-US born, later stage and unmarried status were associated with increased risk of delay. For example, Blacks were at higher risk for delay (HR= 1.2; 95% CI 1.37-1.04) compared to the Hispanic or White (reference) groups. Public insurance (HR = 1.19; 95%CI 1.37-1.04) and no insurance (HR = 1.43; 95%CI 1.85-1.09) was significantly associated with increased delay when compared to private insurance (reference). In patients with neoadjuvant chemotherapy as first treatment, additional significant predictors of delay were age and year of diagnosis.

Conclusion:

Race and insurance status were significant predictors of delay to first treatment in a population of diverse breast cancer patients seeking care at a safety net hospital. Intervention efforts need to target patients at greatest risk for treatment delays.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-09

Title: Predictors of social support among newly diagnosed breast cancer patients seeking care at an urban safety net academic medical center

Ko NY Y, Festa K, Gunn C, Bak S, Wang N, Nelson K, Flacks J, Morton S and Battaglia TA A. Boston Medical Center Women's Health Unit; Boston University School of Public Health and Medical Legal Partnership Boston.

Body: Background:

Disparities in breast cancer care are a worsening problem, requiring effective interventions that seek to address the delivery of high quality cancer care. Evidence from interventions designed to improve timeliness of care routinely identify lack of social support as one of the biggest barriers to care. And, social support is associated with adherence to treatment and survival. This study explores predictors of social support in a diverse population of cancer patients.

Patients and Methods:

This is a secondary analysis of baseline preliminary data from participants enrolled in Project SUPPORT, a randomized controlled comparative effectiveness trial designed to evaluate the impact of patient navigation with or without legal support and services, among women diagnosed with Stages 0-4 breast cancer between 2014-2016. Upon enrollment (within one month of a cancer diagnosis) we administered the Medical Outcomes Survey (**MOS**) of social support to all participants. This validated survey tool addresses functional support, including an overall score (range 0-95) and 4 distinct domains: Emotional/Informational, Tangible, Affectionate and Positive Social Interaction. Using chi-squared and t-tests we compared MOS scores across socio-demographic variables: age, race, language, insurance, health literacy and marital status.

Results:

Of the 103 participants, mean age is 54.5 (SD = 10.6); 56% Hispanic, 19% Black, and 22% White and 2% identified as other; the majority had public insurance 76%; 66% speak English, 21% Spanish and 13% Haitian Creole. Only 36% have adequate health literacy as measured by the BRIEF. Only 32% are currently partnered. The overall mean total score for social support is 75.8 (+/- 23.6), median of 78.9 (range 60.5 – 98.7). Participants scored lowest in tangible support (mean score 67.7 +/- 33.1) and highest in affective support (83.5 +/- 25.8). Non-White participants scored significantly lower across all domains (mean overall MOS score 73.3 +/- 2.6) when compared with Whites (mean overall MOS score 84.5 +/- 4.8, p value = 0.04). There were no differences in MOS scores by language, insurance, literacy or marital status.

Conclusion:

This is the first study to describe social support scores (overall and specific domains) from the validated MOS survey tool among a racially diverse, urban cancer patient population. We found significant differences by race. Studies to identify risks for low social support can help inform future targeted interventions.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-10

Title: Planning and implementation of two regional one-stop breast health centres within a large geographic health authority: Outcomes and quality improvements in health service delivery

Martin LA, Janzen R, Wong F and Doris P. British Columbia Cancer Agency, Surrey, BC, Canada and Fraser Health Authority, Surrey, BC, Canada.

Body: Interrogative examination of wait times for the pathway steps leading to diagnosis, surgical treatment, and oncology consultation for breast cancer within a geographically dispersed publically funded health authority led to advocacy for and establishment of two large one-stop Breast Health Centres (BHCs) within the largest urban cities. One year after publication of the 2000 EUSOMA guidelines for breast cancer diagnosis and treatment, regional breast cancer pathways and wait times did not meet guidelines. Population median wait time from date of first investigation to tissue diagnosis was 2.7 or 5.9 weeks (for clinical presentation or screen presentation respectively), 6.0/7.0 weeks to definitive surgical intervention, and 14.3/11.7 weeks to oncology consultation. Only 39 % of patients were diagnosed initially via core needle biopsy. 5 % of patients underwent immediate reconstructive surgery. Due to wait times for initial imaging (mammogram, ultrasound) at hospital facilities, baseline imaging was often performed at private imaging labs without needle diagnostic capability, and repeated again with a series of wait times at the hospitals - as the initial community images were not accessible to the hospital interventional radiologists. Smaller communities and their hospitals maintained shorter wait times in comparison to the large urban centres. Repeat population based measurement in 2009 indicated wait times were unchanged, and MRI guided biopsy was still not available within the region. Nursing support and patient education re breast cancer prognosis and treatment was not available until the time of oncology consultation.

BHCs were planned, funded by the Ministry of Health, established in physical conjunction with the breast screening programs, and became operational as of early 2012. During the first year of BHC operation, population median wait time from date of first investigation was 2.0 weeks for tissue diagnosis, 6.4 weeks for final definitive surgical intervention, and 9.4 weeks for oncology consultation. 90 % of patients were initially diagnosed via initial core needle biopsy with IHC staining for ER, PR, and HER2. Patients served by the BHCs had nurse navigator support and education from time of presentation. The surgical team overcame regional surgical wait time barriers such as available admission beds by establishing the first comprehensive population-based outpatient mastectomy and reconstruction program in Canada with over 1200 performed to date. These wait times have been maintained in spite of increase in the HA population from 1.2 million to 1.6 million during the project timeline.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-11

Title: Influence of winter season, driving distance, and time on receipt of breast conserving therapy among Iowa urban and rural patients

Wolf CL L, Frankova D and Franko J. Mercy Internal Medicine Residency, Des Moines, IA and Mercy Medical Center, Des Moines, IA.

Body: BACKGROUND: The distance to treatment centers has been reported as a factor influencing the choice of mastectomy over breast conservation therapy (BCT) among breast cancer patients, potentially enhancing disparities for rural patients. We analyzed the impact of crowfly and driving distance to the radiation center and winter season on patient choice of BCT versus mastectomy among breast cancer patients.

METHODS & SETTINGS: Our patient base included 3223 stage 0-3 breast cancer patients treated between 2002 and 2015. Treatment was provided at an established breast center serving urban, suburban, and rural patients in central Iowa.

RESULTS: Crowfly distance (19 ± 22 miles, range 0-160, median 8.7) was consistently shorter as compared to true driving distance (25 ± 27 miles, 0-205, 12.8). One-quarter patients drove over 33 miles (equivalent of 38 minutes). There was tight correlation between crowfly and driving distance (Spearman's $\rho=0.989$, $p<0.001$) and time (0.985 , $p<0.001$).

In univariate analysis, there was no significant difference in crowfly, driving distance, and driving time in minutes to the radiation center between BCT and mastectomy patients (19.3 ± 22.5 vs 18.4 ± 21.6 miles, $p = 0.317$; 25.1 ± 27.5 vs 24.4 ± 27.0 miles, $p = 0.585$; 29.9 ± 25.3 vs 29.5 ± 24.9 minutes, $p = 0.664$).

Receipt of BCT was not predicted by crowfly or true driving distance, driving time, or season. There was no interaction between distance, winter season, and drive time. Receipt of BCT was more likely with older age (OR=1.02 per year of age, $p<0.001$) and later year of treatment (OR=1.05, $p<0.001$).

CONCLUSION: Driving distance, time, and crowfly distance correlated tightly and do not influence patient choice of surgery type for breast cancer in our population. Despite a substantial rural population, driving distance, driving duration, and climate did not significantly influence patients' decisions for mastectomy versus BCT.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-12

Title: Breast cancer clinical trials: Patient experiences, preferences, and insights into increasing participation

Saxton C, Amsellem M and Buzaglo J. Cancer Support Community, Washington, DC.

Body: Clinical trials offer breast cancer patients access to the most innovative treatments, high levels of care and hope for a better future. Yet, fewer than 5% of individuals with breast cancer participate in clinical trials nationally. Education is commonly identified as a target for intervention to increase rates of participation. In late 2015, the Cancer Support Community (CSC) conducted an online survey assessing cancer clinical trials to inform development of an educational program aimed to increase clinical trial awareness and participation. Results from this survey highlighting the experiences, beliefs, and preferences of breast cancer patients are reported here.

239 individuals with breast cancer (47.2% of CSC's national online survey of cancer patients) responded to survey questions assessing their experiences, knowledge, beliefs, and preferences about cancer clinical trials. Most respondents were female (99.6%), Caucasian (84.9%), and averaged 59.6 years old (s.d. =9.7). 21.4% were diagnosed with metastatic breast cancer, and 22.4% had experienced a recurrence.

45% were currently receiving treatment and 75.2% were in remission. Nearly all (97.5%) had health insurance. 106 received treatment at an academic or comprehensive cancer center.

Most (87.5%) had heard of clinical trials pre-cancer, however, there was a wide range of beliefs about the purpose and potential benefits of and barriers to trial participation. While 35.8% had participated in trials, 57.9% had never considered participating in a clinical trial. 53.6% had discussed clinical trials with their healthcare team during treatment. Notably one quarter reported not discussing this at all. Of those having this discussion, 59.2% reported discussions were initiated by the physician, 13.6% by nurse or other healthcare provider, and 17.6% by the patient. During this discussion, only 19% felt that their goals and concerns related to clinical trial participation were meaningfully addressed. Not surprisingly, 95.9% reported more time with their doctors to discuss clinical trials would be "somewhat" or "very" helpful for aiding decision-making.

Respondents reported decision-making tools and resources that would be helpful to them. 97.4% reported that a website which included clinical trial information would be "somewhat" or "very helpful" in identifying clinical trials or aiding decision-making, yet such a resource was shared with only 27.0%. 70.5% reported that speaking directly with another patient who had participated in a clinical trial would be helpful; yet this was only offered to 8.8% of patients.

Based on these findings CSC with partner organizations is developing a series of products and programs to educate individuals who may be eligible for clinical trials. The survey data yields insight into the contents of a successful education and support program around clinical trials and highlights the necessity to provide a comprehensive program that addresses patient preferences for more time to discuss clinical trials with the oncology team and with other patients who had elected to participate in a clinical trial.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-13

Title: Community breast navigation improves breast cancer screening in Hartford, CT

Banks RM M and Stevenson CE E. UConn Health, Neag Comprehensive Cancer Center, Farmington, CT.

Body: Purpose The Community Breast Navigation Program (CBNP) through UConn Health (UCH), in partnership with Susan G. Komen of CT and Community Health Services, Inc. (CHS) in Hartford, CT attempted to address disparities in breast cancer screenings by embedding a Community Breast Navigator (CBN) in the adult medicine clinic of CHS from September 23, 2015 until April 25, 2016. The CBN's principle roles are to provide breast health education, streamline screening scheduling, and follow-up to reschedule missed appointments. The program was implemented to combat barriers to access which cause low rates of mammogram screening. In the United States in 2013, only 37.1% of uninsured women received mammogram screenings compared to 70.4% of insured women. The community breast navigator aims to capture uninsured and underinsured women in an underserved community to improve mammography screenings and early detection of breast cancer.

Scope The objective of the CBNP is to service uninsured and underinsured African-American and Latino women age 40 and over. The mammograms and ultrasounds are offered free of charge to the patient. The costs of the screenings are paid by private donations to UCH. The patient is also offered a gas card or one-day bus pass to provide transportation assistance.

Methods The CBN educates women on the importance of maintaining breast health through breast screenings, clinical breast exams, and self-breast exams and contacts patient access and radiology departments at UCH to schedule screenings. The patient is reminded of the mammogram screening 1-3 days before the appointment. If the patient fails to attend the appointment the CBN contacts the patient and UCH to reschedule the screening.

Results Within 7 months at CHS, 57 (57.6%) of the 99 women referred in the study period received recommended breast screenings. 75.4% of women attended the breast screening with no cancelled appointments, 17.5% of women attended after one cancelled appointment, and 0.7% attended after two cancelled appointments. In CT in 2014, 54.1% of uninsured women received mammogram screenings; through the CBNP 62.2% of uninsured women received mammogram screenings, an improvement of 8.1% in our population. Also, transportation assistance was provided to 32% of patients.

Conclusion To decrease barriers and increase access the CBN scheduled and rescheduled breast screenings. Mammograms costing \$478 for 2D and \$518 for 3D imaging were offered at no cost and gas and bus vouchers were provided to patients. The CBNP addressed common barriers which are cost, transportation, and scheduling to improve mammography screenings and early detection of cancer. Not receiving breast screening was attributed to out-of-service phone numbers, unanswered phone calls and decisions to receive breast screening elsewhere due to the proximity or familiarity with another imaging center. This program should be modeled in health centers and underserved communities to improve mammography screening and close the gap between insured and un/under-insured patients. The most important concepts for the CBNP are to alleviate the patient's barriers and provide a seamless process to receive breast screening.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-10-14

Title: Breast cancer provider delay after the 2011 triple disaster in Fukushima, Japan

Ozaki A, Leppold C, Tsubokura M, Sawano T, Tsukada M and Ohira H. Minamisoma Municipal General Hospital, Minamisoma, Fukushima, Japan.

Body: Introduction

Timely diagnosis and treatment is an indispensable part of breast cancer management. Delay of this process, also known as provider delay, can result in a deteriorated prognosis of affected patients. Although it has been suggested that disasters can impact cancer care and extend provider delay, there is little information available on long-term trends of breast cancer provider delay in post-disaster settings.

So-so district of Fukushima prefecture, Japan, experienced an earthquake, tsunami and the Fukushima Daiichi Nuclear Power Plant Accident in 2011. So-so district has areas falling within the mandatory, voluntary and non-evacuation ordered zones. Due to a long-term shortage of medical staff and closure of medical institutions post-disaster, patients with breast cancer may have experienced longer provider delay in this area.

Objectives

To compare provider delay of breast cancer patients and elucidate contributing factors to delay pre- and post-disaster, in an area severely affected by Japan's 2011 triple disaster.

Methods

We retrospectively investigated data of newly diagnosed breast cancer patients who undertook first medical consultation at the two main cancer centers in the non-evacuation ordered zone of So-so district from 2005 to 2016. Sociodemographic and clinical information was collected from medical records. The main outcome measure was median (days) from first medical consultation to start of breast cancer-specific treatment, pre- and post-disaster, using Mann-Whitney U test. Multivariate linear regression was then conducted to identify any factors which contributed to extended provider delay before and after the disaster.

Results

A total of 157 pre-disaster patients and 121 post-disaster patients were included in the study. There was no significant difference in the interval of median days of first medical consultation to start of first treatment pre- and post-disaster (40 vs. 39, $p=0.82$). Although diagnosis was made in a shorter interval post-disaster compared to pre-disaster (11 vs. 14, $p=0.01$) with significantly smaller median number of biopsies (1 vs. 1, $p=0.001$), this post-disaster improvement in diagnostic process was offset by deferred start of treatment after diagnosis (26 vs. 22, $p=0.008$). Among the pre-disaster patients, cancer detection by breast cancer screening program ($p<0.001$), being engaged in full-time job ($p<0.042$), and number of biopsies before diagnosis ($p=0.005$) contributed to longer provider delay in multivariate regression. However, consultation from other medical providers ($p=0.03$) was the only factor which significantly contributed to extended delay post-disaster, after controlling for multiple variables.

Conclusion

There was no significant increase in provider delay among breast cancer patients post-disaster. However, a median interval of 39 days from first medical consultation to start of treatment is much longer than other high-income countries, and shows much room for improvement in future.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-11-01

Title: Prophylactic contralateral mastectomy - A valid choice?

Subramanian A, Cant G, May S, Jenkins V and Lesley F. East Sussex Healthcare NHS Trust, Eastbourne, East Sussex, United Kingdom and SHORE-C, Brighton and Sussex Medical School, Brighton, East Sussex, United Kingdom.

Body: Whilst the data on patient satisfaction following prophylactic mastectomy and reconstruction in those with a high risk of breast cancer is mostly positive, surgical and nursing opinion regarding contralateral prophylactic mastectomy (CPM) without reconstruction for symmetry +/- risk reduction has a limited evidence base.

Anecdotal experience of UK practice suggests opinion varies enormously. Some surgeons would offer CPM as a method of achieving symmetry whilst some would only perform this if the remaining breast was deemed "problematic" (eg very large or high residual risk). Some surgeons would not countenance removing a "Healthy breast" under any circumstances but would happily reduce a "healthy" breast to achieve symmetry or offer their patients a lengthy reconstruction with the potential complications attached.

This study was designed to assess the variance in opinion towards CPM in breast units across the United Kingdom from both surgeons and specialist breast care nurses and how this compared to views expressed by a cohort of breast cancer patients. 3 questionnaires were designed to canvas opinion from breast surgeons, breast care nurses and members of 3 patient groups who had a range of procedures including reconstruction. This was designed to explore attitudes to CPM as a method of achieving symmetry and potential barriers in those without a perceived high risk of breast cancer. The clinicians questionnaires were distributed to members of the Association of Breast Surgeons of Great Britain and Ireland and the patient questionnaire via 3 web groups.

Respondents: 325/340 patients, 80/709 nurses, 126/588 surgeons.

When the patients were asked, only 50% felt all surgical options were discussed and only 37% felt they were given equal weight. 34 patients had a bilateral mastectomy and 13 patients a prophylactic CPM whereas a further 15 (30%) and 4 (23.5%) respectively wanted one. Out of the patients who had a "healthy" breast removed 54% were to reduce risk and 46% were to achieve symmetry.

84% of surgeons felt that where appropriate breast conserving surgery and mastectomy were discussed equally but over 40% would never discuss or perform prophylactic mastectomy in either the immediate or delayed setting for any reason. 65% of nurses felt CPM should only be considered if broached by the patient and 23% didn't know what their surgeons views on the subject were.

From the nurses perspective if a patient requested a reconstruction 18% felt a psychologist should be involved which rose to 70% if a CPM was considered. For the 60% of surgeons who would perform this procedure 30% didn't involve anyone else in the discussions.

25% of surgeons would offer a CPM for those without a large remaining breast which rose to 40% (22% of nurses) if the breast size was larger. 38% would offer the procedure for a patient with a high genetic risk or a high level of worry independent of proven risk.

32% of surgeons and 16% of nurses felt that the pendulum had swung too far towards reconstruction being the gold standard of achieving symmetry.

Our finding support a heterogeneous approach to CPM in UK practice and that we should in fact be offering this procedure routinely to appropriate patients as an alternative to reconstruction to achieve symmetry independent of genetic or perceived risk.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-11-02

Title: Women's experiences with a decision aid for neoadjuvant systemic therapy for operable breast cancer

Zdenkowski N, Herrmann A, Hall A, Boyle FM M and Butow P. Calvary Mater Newcastle, Newcastle, NSW, Australia; University of Newcastle, Newcastle, NSW, Australia and University of Sydney, Sydney, NSW, Australia.

Body: Background: Neoadjuvant systemic therapy (NAST) is a treatment option for selected patients with highly proliferative and/or large operable breast cancer. Whilst survival outcomes are equivalent between up-front surgery and NAST, the decision about treatment sequence can be difficult due to complexity and perceived urgency of the decision. Patients may value the outcomes of these options, such as down staging and prognostication, differently. Involving patients in decisions about their healthcare reduces anxiety, increases quality of life and satisfaction with care. Decision aids can improve patient involvement in health care decisions, but one is not available for the decision about NAST.

Aims/Methods: We conducted a prospective, single-arm pre-post study to evaluate a custom-designed decision aid developed for women who have been offered NAST. Eligible patients were: female; aged ≥ 18 years; diagnosed with an operable invasive breast cancer; considered for NAST with curative intent. Here, we report on the grounded theory qualitative analysis of a convenience sample of 16 semi-structured phone interviews to explore patient experience with this decision aid.

Results: Participants' median age was 52 (IQR=41-63), median time since breast cancer diagnosis was 5 months (IQR=2-8). Most were married or living with a partner (81.3%) and had a University level degree (68.8%). Patients perceived the decision aid to be useful for becoming more informed and involved in deciding on NAST. Specifically, the decision aid enhanced patients' understanding of their type of breast cancer and the treatment options available to them by summarising and extending the information they received during the consultation with their doctor. Some women perceived the included graphs and statistics to be particularly helpful to understand potential risks and benefits of their treatment options. All patients described the provided information as reliable, relevant and tailored to their needs. They found the decision aid easy to understand and balanced (not in favour of NAST or surgery). The amount of the information provided was seen to be just right. Most women received the decision aid after the initial consultation with their surgeon and perceived this as the right delivery timing. Reading and rereading the decision aid at home in between two consultations allowed women to easily integrate the decision aid into their care. They appreciated the opportunity to reconsider their options after consulting their doctor. A number of women reported that their family members used the decision aid as well and thus became more informed and involved in the decision making process. Some women took the decision aid to the next consultation with their doctor to discuss their preferences and concerns further. All patients followed their doctors' treatment recommendation. The decision aid seemed to confirm but not change women's decisions on NAST.

Discussion: These initial results suggest that this decision aid is a useful tool to assist breast cancer patients' involvement in the decision about NAST. A quantitative analysis of the decision aid's acceptability, feasibility and efficacy will be reported subsequently.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-11-03

Title: Use of real-time patient reported outcomes to improve performance in breast surgery

Whitacre EB B and Moore MN N. Breast Center of Southern Arizona, Tucson, AZ and University of Arizona School of Public Health, Tucson, AZ.

Body: Background: Patient reported outcomes (PRO's) are important to the new health care delivery system but can be difficult to obtain, especially in real-time. The purpose of this project was to demonstrate that 1) An office based iPad patient survey mechanism can obtain PRO's effectively and efficiently; 2) Real time reporting of outcomes to a national database can identify potential gaps in care compared to a peer group of surgeons; 3) Rapid reporting can be used to assess the impact of changes in clinical practice.

Methods: The American Society of Breast Surgeons has a HIPAA secure quality-reporting program for breast surgery which has a parallel secure web site to collect PRO's for patients undergoing breast surgical procedures. Based on patient responses, the physician site reports aggregate PRO's for mastectomy, lumpectomy and breast biopsy, comparing each physicians results with his/her peers. The existing patient survey site utilizes an email invitation and response system, but has only a 34% response rate. Review of the existing data before beginning the project showed that the lowest score for the participating surgeon (EBW) was in response to the question following mastectomy: ***"During your office visit, did your surgeon or your surgeon's staff discuss what to expect with care and recovery after surgery?"*** with an 83% positive response compared to 88% for the aggregate in 2014. To address this, the following clinical changes were made beginning in 2016: 1) Additional emphasis on the expected post op course during initial cancer talk with the patient, 2) a phone call by the surgeon 1-2 days before mastectomy to address any additional questions, 3) a phone call by the surgeon 1-2 days after surgery. In addition, in order to improve the patient response rate, the patient survey was adapted to an iPad, allowing patients to complete the survey in the office at the time of their post operative visit.

Results: The iPad system resulted in a 98% response rate compared to 34% for the email based system. Median time to complete the survey was less than 2 minutes and required no additional staff time, office space, or equipment (other than the iPad itself.) Most patients were very pleased with the iPad platform, although some older patients had difficulty seeing the screen or navigating the touch screen. The high response rate resulted in a very rapid change in the reported PRO's, showing within less than 4 months, that the clinical changes focusing on patient concerns over post operative mastectomy care were successfully addressed, with an increase from 83% to 94% satisfaction for that particular outcome measure.

Conclusion: This study demonstrates that an office based iPad patient survey, linked to a national quality database reporting surgical care, can be used to identify gaps in care, and can then be used to rapidly assess the impact of implemented clinical changes. This is not possible with other national web sites attempting to report quality (e.g. Physician Compare, Health Grades, Vitals), and should be considered for future programs to improve reporting and actionability of PRO's.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-11-04

Title: Adapting evidenced based strategies for effective communication in cancer genetic counseling

Joseph G, Guerra C, Cheng JKY and Lee R. University of California, San Francisco, San Francisco, CA.

Body: As genetics and genomics become part of mainstream Medicine, these advances have the potential to reduce or exacerbate health disparities. Hereditary breast and ovarian cancer risk services are becoming more accessible for low-income individuals, due to the Affordable Care Act of 2010, increased coverage by state Medicaid programs, and decreased costs of testing. Yet access alone is not sufficient to ensure high quality genetic counseling and appropriate testing. Gaps in effective communication are widely recognized as a major contributor to health disparities. The purpose of this study was to describe current communication between cancer genetic counselors and low-income English-, Spanish- and Cantonese-speaking patients in safety net settings, and to assess communication effectiveness from the patient and counselor's perspectives.

Methods: Inductive qualitative methods were used, including observations and audio recording of cancer genetic counseling sessions (n=170), stimulated recall interviews with patients (n= 58), semi-structured interviews with observed genetic counselors (n=10) at two public hospitals.

Results: We identified a fundamental mismatch of patient information needs and information provided by counselors. Components of communication that contributed to this mismatch and resulted in ineffective communication include: (1) provision of information that lacks relevance for the patient; (2) provision of too much information; (3) conceptually difficult presentation of information; (4) imprecise and non-interactive discussion of screening and prevention options. Despite counselors' awareness that they were not always effectively communicating with their limited English proficient and limited health literacy patients, they reported lacking effective tools to draw upon. To address these findings, we adapted evidenced based strategies developed in other medical settings, such as teach-back, plain talk, and proven risk communication methods, to the cancer genetic counseling context. In a pilot, counselors learned about these strategies in a four-hour workshop, and then spent two months practicing in clinic before we began observing them again. Preliminary results of the pilot indicate that counselors are able to apply these strategies to improve patient comprehension and engagement.

Conclusions: Our findings indicate a need to transform the standard model of genetic counseling communication to more effectively meet diverse patients' informational and emotional needs. Particularly for pre-test counseling, counselors need to adapt to the communication needs of the increasingly diverse patients who now have access to hereditary breast and ovarian cancer testing, including the many with limited health literacy and limited English proficiency. Counselor training in communication tools proven effective for patients of low health literacy in other fields should be integrated into training curricula.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-11-05

Title: Reliability and acceptance of e-based survey instruments for measuring patient reported outcomes (PRO) in breast cancer patients: First results of the ePROCOM study

Wallwiener M, Simoes E, Hartkopf AD, Taran F-A, Keilmann L, Sickenberger N, Stevanovic S, Belleville E, Ladra C, Schneeweiss A, Wallwiener D, Brucker SY and Graf J. University Hospital Tuebingen, Tuebingen, Germany and University Hospital Heidelberg, Hospital for General Obstetrics and Gynecology, Heidelberg.

Body: Introduction

Especially in oncology patients, Patient Reported Outcomes (PRO) play an increasingly important role to measure subjectively perceived health status and treatment effects. At the moment, paper-based surveys of PRO still predominate (pPRO); in recent years, data on patient-relevant endpoints is being increasingly collected electronically (ePRO). The aim of the study was to analyze the acceptance of an ePRO-survey tool in breast cancer patients within the PRAEGNANT multicenter trial. Furthermore, it should be considered, whether differences in response behavior between pPRO and ePRO can be identified (reliability check).

Materials and Methods

ePROCOM (**P**atient **R**eported **O**utcomes and **C**ompliance Anaysis) was conceptualized as a monocenter, randomized, parallel-group, cross-over study. Female patients with diagnosis breast cancer aged more than 18 years were included. We randomized the patients into one of two study arms. In study arm A the patients are first asked to use the electronic, web-based tool to document the patient questionnaire (EORTC QLQ C-30 and FACT-B). Afterwards the patients were asked to fill and evaluate the paper-based questionnaires accordingly, followed by evaluation of usability, acceptance and capability. In study arm B the course varies by meaning that paper-based evaluation will be followed by the assessment of electronic data capture.

Results

N=110 patients with breast cancer in adjuvant or neoadjuvant situation completing the study during an outpatient visit at the University Hospitals in Tuebingen and the National Cancer Centre Heidelberg (average age: 52.4). In most patients, there were no differences in terms of acceptance between pPRO and ePRO. Only in some older patients with a lower quality of life hurdles for ePRO could be identified, because of lower acceptance rates. We could not find significant differences in response behavior between pPRO and ePRO.

Discussion

Because no differences in response behavior could be identified, the tool can be define as reliable possibility to measure patient reported outcomes. E-PRO surveys appear to be suitable for use in breast cancer patients. However, there is a need of support in older and more ill patients, to participate form the technical capabilities of ePRO.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-11-06

Title: Development and application of whole processing management for patients with breast carcinoma: A novel management strategy for breast health

Chen J, Yu K, Liu G, Shao Z and Wu J. Fudan University Shanghai Cancer Center, Shanghai, China and Fudan University Shanghai Medical College, Shanghai, China.

Body: The reformation on the management strategy of patients led by medical institutions could show the real value of medical services and match the needs of both patients and physicians. A novel patient management strategy, the whole processing management mode, was developed in Department of Breast Surgery, Fudan University Shanghai Cancer Center. Based on the online tools, the e follow-up Wechat public platform, the department provides several humanistic medical services in every aspects of breast health management, such as education, screening and diagnosis, multi-disciplinary comprehensive treatment and follow-up, including the remote medical reports reading. After the application of the novel patient management mode, regular appointments before seeing doctors, triage on-demand are realized. Between Jun-2015 and March-2016, a total of 7,668 person-time patients made the reservation for chemotherapy and over two thousand patients made the reservation for endocrine therapy prescription through the online approach. The total volume of out-patient clinic was 169,246 person-time in 2015, gained a 26.8% increase compared with in 2014. However, the queuing time for consultation was decreased by 28.8% in 2015. The doctor-patient relationship is extended online and the follow-up rate is improved. Between Jun-2015 and December-2015, a total of 823 patients online submitted the medical reports during follow-up period, among those, four patients were found disease relapse or metastasis and were call back to the hospital for further treatment. The novel mode may promote the cooperation among the multi-disciplinary comprehensive treatment teams, realize the hierarchical medical system among the department, and provide guarantee for the clinical and basic research based on the principle of precision medicine.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-12-01

Title: Value-based approach to treatment of HER2-positive breast cancer: Examining the evidence

Nixon NA A, Hannouf M and Verma S. Tom Baker Cancer Center, Calgary, AB, Canada and Ivey School of Business, Western University, London, ON, Canada.

Body: Outcomes of HER2-positive breast cancer have improved significantly with use of targeted therapies. Survival in both early (EBC) and metastatic breast cancer (MBC) has improved along with gains in quality of life. With increasing costs of cancer care, it became imperative that health systems evaluate cost-effectiveness and value provided by new therapies.

Methods: We conducted a review of 'value' utilizing the ASCO 2015, ASCO 2016 (revised) and ESMO framework for all currently available HER2 targeted therapies. We performed a systematic review of cost-effectiveness analyses (CEAs) of these therapies across the neoadjuvant, adjuvant, and metastatic disease settings. We included economic evaluations from published literature and government agencies involved in drug-approval assessments from NICE (UK), pCODR (Canada), and PBAC (Australia).

Results: 22 studies evaluating 1-year of trastuzumab (H) in EBC were identified. Of these, 17 found the regimen cost-effective (CE). Three of 22 plus an additional 1 evaluated 9 weeks H, all of which found it CE. NICE and PBAC determined adjuvant H to be CE, consistent with clinical benefit (Table 1). There are currently no academic CEAs of neoadjuvant pertuzumab (P). It has been evaluated in drug-approval processes by NICE and pCODR, both finding cost-effectiveness highly uncertain. In MBC, 6 studies evaluating H for first line were identified. The combination with chemotherapy was CE in 3 of 4 studies, whereas monotherapy and combination with anastrozole were not. A total of 9 studies evaluating lapatinib for MBC were identified. While it was CE combined with capecitabine for second line, in all other combinations it was not. Two studies evaluating P for MBC did not find the regimen CE, despite significant clinical benefit. However, PBAC considers the regimen CE and pCODR recommended funding based on net clinical benefit, whereas NICE did not. No academic CEAs of trastuzumab emtansine (T-DM1) in the literature were identified however cost-effectiveness in second line has been evaluated by pCODR, NICE and PBAC. All groups found it not CE, even with very high clinical benefit (Table 1).

Table 1: ASCO Net Health Benefit (NHB), modified NHB (mNHB) and ESMO Magnitude of Clinical Benefit Score (MCBS) for landmark trials in HER2+ breast cancer compared with cost-effectiveness

Study	Setting	Regimen	NHB	mNHB	MCBS	Cost-Effective
NeoSphere	EBC (Neoadjuvant)	DH+/- P->surgery->FEC	NA*	NA*	NA*	-
TRYPHAENA	"	DCH+P->surgery	NA	NA	NA	-
NSABP-B31/NCCTGN9831	EBC (Adjuvant)	AC-based chemo +/- H	48	28	A	Y
FinHer	"	FEC based chemo +/- H x 9 weeks	NA*	NA*	NA*	Y
HERA	"	Chemo +/- 1y H	32	26	A	Y
Slamon et al	MBC (1st line)	TH vs T	16	17.7	2	Y
CLEOPATRA	"	DHP vs. DH	32	32	4	N
TanDEM	"	Anastrozole +/- H	22	13.9	3	N
Johnston et al	"	Letrozole +/- Lapatinib	55	13.6	1	N
EMILIA	MBC (2nd line)	T-DM1 vs. lapatinib + cape	42	46.4	5	N
EGF100151	MBC (>/=2nd line)	Cape + lapatinib vs. cape alone	16	29.4	4	N

* = Not significant

Conclusion: While there is consistent value provided by Her2 targeted therapies, there is generally lack of support for these in MBC based on cost-effectiveness analysis. We need to work towards a model that integrates value, clinical benefit and cost to implement new therapies in cancer, including HER2 positive breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-12-02

Title: Cost-effectiveness analysis of adding neoadjuvant chemotherapy with pertuzumab, in patients with HER2+ breast cancer in Spain

Abanell J, Ciruelos E, De la Haba J, Martín M, Muñoz-Molina B, De Salas-Cansado M and Colomer R. Hospital del Mar, Barcelona, Spain; Hospital Universitario 12 de Octubre, Madrid, Spain; Hospital Universitario Reina Sofía, Córdoba, Spain; Roche Farma S. A, Madrid, Spain; Hospital Universitario La Princesa, Madrid, Spain and Hospital Universitario Gregorio Marañón, Madrid, Spain.

Body: **OBJECTIVES:** Given the increase resource constriction of the healthcare systems, efficiency analyses are become more frequent to give additional information to help decision makers in the adoption of new interventions that have already proved clinical value. The use of a neoadjuvant chemotherapy regimen including pertuzumab, trastuzumab and docetaxel (PTD) has demonstrated to improve pathological complete response (pCR) in patients with HER2+ breast cancer (BC). The objective of this study was to estimate the cost-effectiveness of PTD vs. TD in Spain.

METHODS: Clinical outcomes from the NeoSphere trial were combined with local data regarding the use of resources associated to patients' follow-up and management of recurrences in a Markov model. Markov models are commonly used to represent the evolution of cancer patients, the effect on clinical outcomes and costs of alternative treatment strategies. In this study, patients with HER2+ BC were modeled through six different health states (event-free, locoregional-relapse, remission, metastatic-relapse [1L], metastatic-relapse [2L], and death) in two cohorts receiving PTD or TD. Long-term effects were estimated by fitting a log-logistic distribution (other techniques were also explored) to event free survival (EFS) data from the NeoSphere study (prevention of 5% relapses at 5 years) combined with utility estimates from the literature. Other transition probabilities as well as unit costs were also obtained from the literature and validated by a 15-oncologists Delphi panel, as well as the estimation of the patients follow-up and recurrences costs. The effectiveness was expressed in terms of quality adjusted life years (QALY) and the analysis was performed from the perspective of the Spanish National Healthcare System (NHS) over a lifetime horizon (a 3% annual discount rate was used for both costs and effects). Deterministic and probabilistic sensitivity analyses (PSA) were performed to assess the robustness of the model results and the influence of different input parameters.

RESULTS: PTD was associated to higher effectiveness than PT (14.007 and 13.447 QALY respectively) and lower lifetime costs (€141.070 € and €148.299 € respectively). PTD was also a dominant option over PT when pathological complete response results from the NeoSphere study were combined with EFS data from the CTNeoBC analysis to estimate long-term results. Both the deterministic and probabilistic sensitivity analyses confirmed the robustness of the results, showing that PTD was a dominant option in more than 90% simulations in the PSA.

CONCLUSIONS: Beyond the better long-term health outcomes, the combination of PTD would also result in lower lifetime costs for the Spanish NHS, showing that adding pertuzumab is a dominant strategy in HER2+ BC patients receiving neoadjuvant therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-12-03

Title: Evaluation of women with *BRCA* mutations and breast cancer tested at an NCI designated comprehensive cancer center: A cost of illness estimation

Biskupiak JE E, Telford C, Yoo M, Unni SK K, Ye X, Deka R, Brixner DI I and Stenehjem DD D. College of Pharmacy, University of Utah, Salt Lake City, UT; AstraZeneca Pharmaceuticals, Gaithersburg, MD; Program in Personalized Health Care, University of Utah, Salt Lake City, UT and Huntsman Cancer Institute, Salt Lake City, UT.

Body: Objectives: Little is known regarding the impact of mutation status on the costs of breast cancer care. This study sought to estimate health care charges (all-cause, BC related, other cancer related and non-cancer related) among women with breast cancer (BC) and *BRCA* mutations (*BRCAm*) in terms of *BRCA* status, HER2 expression status, ER/PR status, treatment patterns, serious adverse events related to the cancer or its treatment, and cancer treatment period.

Methods: Adult women with invasive BC diagnosed from 1995-2014 and tested for a *BRCAm* were identified from the Huntsman Cancer Institute tumor registry (Salt Lake City, Utah) and via chart review. Patients with available charge data were included in the study. Patients were categorized by mutation status, receptor status and initial treatment setting. Charges were categorized as inpatient, outpatient and pharmacy (both anticancer and other medication) related charges as well as by type of service (diagnosis, surgery, reconstruction, radiation, office visit, and medication). Descriptive statistics were used to describe mean (SD) charges. Wilcoxon Rank-Sum test was used to compare health care charges.

Results: There were 816 women with BC who underwent *BRCA* testing and had available charge data. There were 134 women with a *BRCAm* vs 682 with *BRCA* wild type (wt). Age at diagnosis was similar between the two groups, however, *BRCAm* patients had more triple negative BC and higher histologic grade. Total breast cancer related mean (SD) charges were similar between *BRCAm* vs *BRCAwt* (\$86,689 (75,937) vs \$85,843 (97,304), $p=0.19$). Of this a similar amount was due to facilities/ technical and pharmacy (41% each), while the remainder was physician/professional costs. Within pharmacy costs, similar amounts were due to chemotherapy (23%) and biologics (21%). However, while 58% received chemotherapy, only 8% received biologics. Patients seen initially in the neoadjuvant treatment setting (N=148) had higher breast cancer related mean charges than those in the adjuvant setting (N=553; \$117,922 (102,108) vs \$80,061 (90,010), $p<.0001$), while those seen initially in metastatic setting had a mean charge of \$103,525 (135,029). There were 142 HER2+ (ER+/PR+/HER2+ or ER-/PR-/HER2+) and 521 HER2- (TNBC or ER-/PR+/HER2-) breast cancer patients (receptor status unknown in 153 patients). HER2+ patients had higher breast cancer related mean charges than HER2- patients (\$155,858 (122,227) vs \$69,883 (67,642), $p<.0001$). Further, anticancer treatment charges accounted for 53% (\$82,890 (81,269)) of HER2+ costs and 11% (\$7,929 (21,782)), $p<.0001$ of HER2- costs. Biologics accounted for 87% of the former and chemotherapy accounted for 78% of the latter charge.

Conclusions: Mutation status was not associated with higher breast cancer charges. Patients initially seen in the neoadjuvant setting had higher breast cancer charges than those seen in the adjuvant setting. Receptor status (being HER2+) was associated with higher breast cancer charges and this was driven by expenditure on biologics.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-12-04

Title: Is 6 hour monitoring for administration related reactions after first administration of subcutaneous trastuzumab necessary?
A single institution audit

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Body: Background: Subcutaneous (sc) trastuzumab has demonstrated non-inferiority to intravenous (iv) trastuzumab and is preferred by patients and providers. Serious administration related reactions (ARRs) such as hypotension, respiratory distress etc. were not reported in the pivotal HannaH study. However, the summary of product characteristics (SPC) advises that patients should be observed for ARR for 6 hours post the first injection (and 2 hours post subsequent injections), similar to the iv formulation.

Methods: We conducted an audit of patients commencing sc trastuzumab at our institution. Medical notes of each patient were reviewed to record adverse events reported on the day of first administration or at the subsequent visit. In addition all patients were interviewed by telephone and questioned regarding adverse events with first or subsequent injections.

Results: 39 patients were identified, 32 had received prior iv trastuzumab. Patients received a mean of 12 injections. In total patients received 470 sc trastuzumab injections, associated with a recommended 1,096 hours of observation as per SPC. 3 injections (0.6%) were associated with ARR within 24 hours, all on the first cycle. 2 patients (5%) experienced injection site reactions immediately post injection and 1 patient had injection site pain during the injection. 1 patient experienced pyrexia and dry cough 24 hours post injection and was hospitalized for respiratory tract infection. No patient experienced a reaction between 2 and 6 hours post first injection. There were no serious ARRs.

Conclusions: ARRs related to sc trastuzumab are usually immediate, mild and self-limiting. Observing patients for 6 hours post first injection and 2 hours post subsequent injections represents an inefficient use of healthcare resources.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-12-05

Title: Estimating costs of relapsing HER2+ breast cancer in Spain

Colomer R, Ciruelos E, De la Haba J, Martín M, De Salas-Cansado M, Muñoz-Molina B and Albanell J. Hospital Universitario La Princesa, Madrid, Spain; Hospital Universitario 12 de Octubre, Madrid, Spain; Hospital Universitario Reina Sofía, Córdoba, Spain; Roche Farma S. A, Madrid, Spain; Hospital del Mar, Barcelona, Spain and Hospital Universitario Gregorio Marañón, Madrid, Spain.

Body: **OBJECTIVES:** The purpose of this study was to describe the economic impact of recurrences (locoregional or unresectable/metastatic recurrences [MR]) in patients with HER2+ breast cancer (BC) in Spain.

METHODS: A Delphi panel of 15 Spanish oncologists was carried out to define the clinical management (patients' flow and healthcare resources use) of locoregional recurrences (LR) and unresectable or MR in patients with HER2+ BC. Healthcare resources included medication, medical visits, lab and imaging tests, hospitalizations, surgery, adverse events management and treatment of specific metastases (e.g. bone metastases). Additionally, loss of productivity was also estimated. Local unit costs were used in combination with healthcare resources and workdays lost to estimate both the direct and indirect costs of LR and MR in HER2+ BC patients. A deterministic sensitivity analysis was performed to explore the robustness and assess the critical variables of the study.

RESULTS: The estimated distribution of recurrences in HER2+ BC patients was: 90% of MR, 7% of LR and 3% of LR followed by an MR. With respect to MRs, 31% of cases were estimated to receive up to 5 lines of treatment whilst 5% received no treatment (10%, 20% and 21% would receive 2, 3 and 4 treatment lines, respectively, with a median of 3 lines). Treatment regimens including trastuzumab were the most frequently used (in combination with pertuzumab and taxane in 1L, and with other combinations in 4&5L). In 3L, the most common treatment regimen was lapatinib-capecitabine. Total costs, including medical and indirect costs were €235,138, being the direct costs more than 88.7% (€208,682). Both direct and total costs were higher in patients being treated with a higher number of treatment lines, with figures ranging from €38,511 (1L) to €308,869 (5L) and €50,908 (1L) and €358,000 (5L) respectively.

CONCLUSIONS: HER2+ BC patients with a MR were estimated to receive a median of 3 treatment lines and almost one third receives 5 treatment lines. The direct and indirect costs of recurrences are high, with a calculated average total cost of €235,138. Using targeted therapies in earlier stages of HER2+ BC that achieve a reduction of recurrences would achieve significant cost savings in Spain.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-12-06

Title: Saving in clinical trials: A possible challenge for improving health care for breast cancer patients

Taverniti C, Bonfadini C, Pradotto M, Cagnazzo C, Demartini P, Rossi L, Ignazzi G, Arizio F and Beano A. A.O.U. Città della Salute e della Scienza - Breast Unit, Torino, Turin, Italy; University of Turin - San Luigi Hospital, Orbassano, Italy; Istituto di Candiolo, Fondazione del Piemonte per l'Oncologia - IRCCS, Candiolo, Turin, Italy and AOU Città della Salute e della Scienza - CTO, Turin, Italy.

Body: Background: The economic crisis that has characterized recent years has required a careful spending review worldwide and particularly in Italy Health Care System. This resulted in substantial cuts in terms of services and drugs prescriptions. For this reason, it becomes very important to adopt measures aimed to save health care costs, maintaining at the same time the highest standard of health care. The enrollment in clinical trials (CTs) may encourage this goal, making high-cost drugs available.

Methods: We compared the overall expenses for an oncology patient treated with standard therapy (reference: public hospital price list) to a similar patient enrolled in a CT. The cost comparison was made considering a patient affected by Her2 positive breast cancer in three different settings: neoadjuvant, adjuvant, and advanced/metastatic disease. We considered three categories of cost: diagnostic procedures; drug purchase; drug management. All analyses were performed considering an average treatment period and dose, calculated on a middleweight patient.

We take into account the absolute saving in Euro (€) and the percentage one.

Results: The data show a saving in all treatment lines, referring to the analyzed cost groups.

In neoadjuvant setting, the estimated patient standard cost is 92.829,00€; we calculated a saving of 92.451,00€ (93,74%), of which 1.226,00€ for diagnostic procedures charge (65%), 89.205,00€ for drug purchase (100%) and 2.020,00€ for drug management (116%).

In adjuvant setting, where the estimated standard cost is 25.527,00€, we observed a saving of 23.332,00€ (51%): 22.392,00€ (98% of drug purchase) and 940,00€ (54%) for drug management; no saving for diagnostic procedures.

Regarding the metastatic breast cancer treatment, we calculated a saving of 115.158,00€ (88%) compared to 115.571,00€, that means the estimated standard cost. In this case the saving is allocated as follows: 750,00€ for diagnostic procedures (64.5%), 113.508,00€ for drug purchase (100%) and 900,00€ for drug management (100%).

Conclusions: CTs are useful in order to save money in the overall patient management, allowing them to have access to expensive innovative drugs.

Moreover, we have to consider the additional fees provided by Sponsor for each enrolled patient.

This basic model of cost analysis could be used by Institutional Health Care stakeholders for spending review strategies.

The public institutes with the characteristics of Comprehensive Cancer Center are essential to recruiting the needed study population for Sponsored CTs, and the cashed and saved money can be reinvested for improving Breast Cancer patients care.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-01

Title: Determining the breast tumor margin through genomics of the cancer-stromal interaction

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Body: Introduction: Data indicate that breast tumors can enact gene expression changes in the peri-tumoral stroma that remains after surgical removal of the tumor. This tumor adjacent normal tissue harbors genomic changes that reflect early transformation events as well as promote a tumor-nurturing environment making it highly receptive to future cancer development, tumor invasion, metastasis and recurrence. The physical and spatial extent at which abnormal genomic changes penetrate into the surrounding tissue has not been examined. We hypothesized that a genetically defined border exists between tumor altered, disease promoting stromal tissue and normal tissue *based on* tumor imprinted alteration in gene expression and distance from tumor. **Methods:** Samples of tumor and tumor-adjacent histologically normal tissue at 5 mm, 10mm, 15mm, and 20 mm were obtained from 33 patients undergoing mastectomy. Tissues were analyzed for genome-wide mRNA expression using microarrays. An unsupervised NMF analysis for data dimension reduction of tissue clustering, was performed. In order to define a subset of genes that have similar expression in tumor and at least one distance of normal tissue, we defined a tumor signature and a tumor-like gene signature. To define a tumor-like gene signature, pair-wise statistical analysis was performed to isolate genes with a 2-fold change in expression (p .value < 0.01) in at least one distance of normal tissue compared to tumor. Using tumor-like genes, hierarchical clustering was used to separate genes into 7 clusters based on average gene expression levels across tumor and each distance of normal and further examined for functional characterization using PANTHER gene analysis tools. Correlation studies examining each patient's gene expression in normal tissue compared to tumor were carried out. **Results:** The tumor signature is enriched in proliferative genes and pathways related to angiogenesis. The tumor -like signature is enriched with pathways that are tumor promoting such as disruption of basement membrane, migration, and angiogenesis. The gene signature in the 20mm region reveal that the cells have not been transformed but are highly plastic and in a state of flux between maintaining activities of normal cells, tumor suppression and are also pro-transforming of processes involved with proliferation, extracellular re-modeling and EMT. We found key tumorigenic pathways displayed a general decrease of gene expression at distances further from tumor and particularly the 20 mm region displayed an enrichment of pathways necessary for normal tissue maintenance. We found that 50% or more samples within 5mm, 10mm, and 15mm correlate well ($R > 0.5$) with tumor expression compared to only 30% of 20mm tissues. **Conclusion:** Breast tissue devoid of tumor cells is genomically related to tumor, most significantly within 5mm. These genomic changes display a gradient from tumor truncating at 20 mm. The impact of the stromal cell gene profile and pre-malignant altered cells in the remaining stroma after surgery, may be defined by genetic alterations in abnormal stroma that resemble those in tumor particularly at a 5mm distance. This tumor-like signature may be important for making and personalizing surgical and adjuvant therapeutic decisions.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-02

Title: Impact of primary surgery on short-term survival of older breast cancer patients in the UK

Bundred N, Lavelle K, Sowerbutts AM, Pilling M and Todd C. The University of Manchester, Manchester, United Kingdom.

Body: Introduction

Lack of surgery for older breast cancer patients may reduce cancer survival. Previous studies did not adjust for comorbidity and tumour characteristics which affect survival.

Methods

In a prospective cohort study investigating older patients' treatment, survival analyses (mean 3.8 years, 95% CI: 3.69-3.83) was undertaken for 910 breast cancer patients aged ≥ 65 years diagnosed at 22 English hospitals from 1/7/10 to 31/12/12. Primary outcome was breast cancer specific survival. Independent variables included surgery, comorbidity, functional status and tumour characteristics recorded from patient interview (at diagnosis) and case note review. Data analyses included Cox's multiple regression.

Results

Adjusting for tumour stage, comorbidity and functional status, women undergoing primary surgery (n=772) had a third of the hazard of breast cancer death compared to those who did not (n=138) (HR 0.36, 95% CI: 0.20-0.66, p=0.001). The number of observed breast cancer deaths exceeded those expected for participants who did not have primary surgery, were aged ≥ 85 years, steroid receptor negative or had a higher grade or stage tumour. In univariate analysis women aged ≥ 85 years had an increased hazard of breast cancer death compared to 65-69 year olds (HR 4.02, 95% CI: 1.61-10.01, p=0.003). Patients' role in the treatment decisions did not alter whether they received surgery or not; those who were active/collaborative were as likely to get surgery as those who were passive (i.e. left the decision up to the Surgeon).

Conclusions

Surgery for older breast cancer patients reduces the hazard of cancer death by a third, independent of age, comorbidity and tumour characteristics. Surgeons must actively advise surgery for all elderly patients.

Cox's proportional hazards regression of breast cancer specific survival (unadjusted n=906)

Variable	Category	Unadjusted HR	Univariable 95% CI	P Value	Adjusted HR#	Multivariable 95% CI	P Value
Primary surgery	No	(ref)			(ref)		
	Yes	0.32	0.19-0.53	<0.001	0.36	0.20-0.66	0.001
Age group (years)	65-69	(ref)			(ref)		
	70-74	1.53	0.61-3.86	0.364	1.31	0.52-3.34	0.565
	75-79	1.35	0.51-3.54	0.548	1.04	0.39-2.77	0.933
	80-84	2.39	0.92-6.22	0.074	1.72	0.65-4.56	0.272
	85+	4.02	1.61-10.01	0.003	2.61	0.99-6.91	0.053
Grade	1	(ref)			(ref)		
	2	1.37	0.60-3.14	0.453	1.18	0.51-2.71	0.704
	3	4.55	2.01-10.31	<0.001	3.23	1.36-7.65	0.008
ER or PR positive	Yes	(ref)			(ref)		
	No	3.50	2.02-6.08	<0.001	2.75	1.49-5.09	0.001
Tumour Stage	I	(ref)			(ref)		
	II and IIIa	2.25	1.33-3.81	0.002	1.48	0.85-2.57	0.164
	0	(ref)			(ref)		

Co-morbidity (Charlson)							
	1	1.02	0.60-1.74	0.935	0.97	0.56-1.67	0.917
	2+	0.96	0.50-1.84	0.902	0.80	0.41-1.57	0.518
Functional status*	Independent (1-2)	(ref)			(ref)		
	Dependent (3-4)	1.69	0.97-2.95	0.064	1.00	0.53-1.88	0.995

Adjusted for all other variables in table

(Funded by NIHR Programme Grant).

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-03

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-04

Title: Use of an absorbable implant to mark the lumpectomy cavity: Initial report of 300 patients in a multi-center registry database

Kaufman CS S, Cross MJ J, Goyal S, Barone J, Devisetty K, Dekhne NS S, Edmonson D, Gass JS S, Graham CL L, Hong RL L, Patton BJ J, Phillips RF F, Schonholz SM M, Smith LA A, Tafra L, Smith AB B and Dilworth J. University of Washington, Bellingham, WA; Breast Treatment Associates; Robert Wood Johnson / Rutgers University; Exempla St. Joseph Hospital; Staten Island University Hospital; William Beaumont Hospital; Womens and Infants' Hospital; Georgia Breast Care; Virginia Hospital Center; Metro Surgical Associates; Noble Hospital; Lovelace Women's Hospital; Arizona Breast Cancer Specialists; Anne Arundel Health System; Karmanos Cancer Institute at McLaren Flint; Exempla St. Joseph Hospital and Highlands Oncology Group.

Body: Objective:

Increased use of oncoplastic procedures makes it difficult to distinguish the lumpectomy margins from seroma fluid. Many side effects of radiation therapy are due to overestimated treatment volumes due to seroma based planning. A novel 3-dimensional (3D) absorbable device sutured to the lumpectomy tumor bed allows greater precision in targeting of the margins while avoiding normal tissues. A multi-center clinical registry was established to assess use of this device over time. This initial report of the first 300 patients provides insight into patient selection, tumor characteristics, method of implantation and initial outcomes.

Methods:

A bioabsorbable 3D implantable marker with a fixed array of 6 titanium clips was sutured to the site of the excised breast cancer during lumpectomy with or without oncoplastic closure techniques. The framework of the implant resorbs slowly over time, while the clips remain permanently. The marker was utilized for boost or partial breast irradiation (PBI) planning or treatment targeting. To date the registry has accrued 300 patients from 12 centers. Data includes patient demographics, breast size, tumor characteristics, surgical and radiotherapy techniques, cosmesis and follow-up.

Results:

Data on 300 patients with median follow-up 10.4 months was analyzed. Median age was 64.4 years, 83% of women were postmenopausal and 10% had comorbidities. Breast size was evenly distributed between cup size B, C and \geq D. Cancer histology was in-situ (20%), invasive ductal (62%), invasive lobular (9%) and others (10%) measuring T1 (80%) and T2 (20%). Laterality and tumor location within the breast were typical. Most patients had oncoplastic rearrangement (74%) at the time of lumpectomy. Re-excision (including mastectomy for extensive disease) occurred in 14% of patients. Infections occurred in 2% of patients and in one patient the device was removed during surgical debridement of the postoperative infection. No devices have been removed during follow-up due to misplacement or patient-generated concerns. No cancer recurrences have been reported. In most cases, size of the device reflected size of the tumor with the 2X2cm (47%) and 2X3cm (32%) devices used most often. The device was utilized by radiation oncologists for boost or PBI planning and treatment. Data on ease of setup and boost planning is being collected. Early reports regarding cosmetic appearance show a trend for excellent or good cosmesis as judged separately by both physicians and patients (93% and 92%).

Conclusions:

A novel 3-dimensional absorbable device sutured into the tumor bed provides clarity in radiation targeting of tissues at greatest risk while sparing adjacent normal tissues mobilized during surgery. This initial report of a multi-center clinical registry on over 300 patients characterizes patient selection, treatment methods and follow-up. Ongoing collection of additional data, regarding radiation regimen, cosmesis and patient satisfaction, have been accumulated and provide insight regarding clinical attributes of this implantable marker.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-05

Title: Multicentre observational study evaluating why mastectomies are advised by UK multi-disciplinary teams

Singh JK K, McEvoy K, Marla S, Wilcox M, Rea D, Hallissey MT T, Francis A and West Midlands Research Collaborative. University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom; University Hospitals of Coventry and Warwickshire NHS Foundation Trust, Coventry, United Kingdom; Royal Wolverhampton Hospitals NHS Trust, Wolverhampton, United Kingdom and Independent Cancer Patients' Voice, United Kingdom.

Body: Background: Marked variation in mastectomy rates exists across the UK. Identification of variation in practice is a key step towards standardisation of service. The rationale for advising mastectomy by multi-disciplinary teams (MDTs) has not been previously explored in the UK. The main aim of this multicentre observational study was to describe current practice in MDT decision-making for patients undergoing mastectomy. A secondary aim was to determine utilisation of neoadjuvant therapies.

Methods: A multicentre, protocol-driven, prospective cohort study, led by trainees of the West Midlands Research Collaborative was performed during July and September 2015. Data was collected securely using Research Electronic Data Capture. Inclusion criteria were: women >18 years undergoing mastectomy for in situ/invasive disease; presenting with symptomatic or screen detected disease; performed as a primary procedure or following failure of breast conserving surgery (BCS); with or without immediate breast reconstruction (IR).

Results: A total of 1776 patients (1823 mastectomies; 47 bilateral procedures) from 68 units were included. Median age was 63 years (range 20-99). In total 481 (26%) IRs were performed; median IR rate was 22% (range 0-67%).

Mastectomy was advised by the MDT in 1402 (77%) cases. Reasons for advising mastectomy are shown in Table 1.

Table 1. MDT rationale for advising mastectomy

Rationale	Number of mastectomies	Proportion (%)
Large tumour to breast size ratio making BCS unsuitable	530	29.1
Multi-centric disease on imaging	372	20.4
Extensive malignant microcalcification	179	9.8
Previous radiotherapy (Breast/Mantle)	163	8.9
Requiring further surgery for positive margins following BCS	158	8.7
Central tumour	113	6.2
Large primary tumour, patient not suitable for neoadjuvant endocrine or chemotherapy treatment	112	6.1
Neoadjuvant therapy failed to downsize tumour to allow BCS	88	4.8
Neoadjuvant therapy apparently successful but mastectomy advised anyway	79	4.3
Family History-High Risk	51	2.8

In total 153 patients with oestrogen receptor positive (ER+) tumours were offered neoadjuvant endocrine treatment (NET); 131 (86%) received treatment. A total of 293 post-menopausal women with uni-focal, ER+ tumours, >20mm were *not* offered NET; mastectomy was advised by MDTs in 202 patients and the rationale for advising mastectomy in 173 patients (86%) was large tumour to breast size ratio.

In total 104 patients with Human Epidermal Growth Factor Receptor 2 over-expressing (HER2+) tumours were offered neoadjuvant chemotherapy and trastuzumab (NACT); 89 (86%) received treatment. A total of 88 women <70 years old with HER2+ tumours, >20mm were *not* offered NACT; mastectomy was advised by MDTs in 75 patients and rationale for advising

mastectomy in 45 women (60%) was large tumour to breast size ratio.

Conclusions: Although most mastectomies are advised for large tumour to breast size ratio, there is inconsistency in the utilisation of neoadjuvant therapies with many potentially eligible patients with large tumours not being given the opportunity to be downsized. Application of standardised recommendations for neoadjuvant treatment resulting in increased and appropriate use of neoadjuvant therapies could reduce the number of mastectomies advised by MDTs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-06

Title: Development and validation of a nomogram predicting pathological axillary status (ypN0 vs. ypN+) in a subgroup of patients converting from cN+ to ycN0 through neoadjuvant therapy (NAT) – A transSENTINA substudy

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Body: Background: Particularly among patients converting from cN+ to ycN0 status through neoadjuvant therapy (NAT) the optimal method and extent of axillary staging is unclear. The aim of this analysis was to develop a nomogram predicting the probability of positive axillary status (ypN+) after PST among these patients based on clinical and pathological parameters.

Methods: Patients converting from cN+ to ycN0 due to PST included in a prospective study (SENTINA, Arm C) were included. Univariate and multivariate analyses were carried out to evaluate the association between 14 clinical/pathological parameters and pathological axillary status (ypN0 vs ypN+) using logistic regression models. Model accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were assessed applying leave-one-out cross-validation (LOOCV) and ROC analyses. Different cut-points were evaluated. Calculations were performed using the SAS Software (Version 9.4, SAS Institute Inc., Cary, NC, USA.).

Results: Arm C contained 553 patients, 369 patients were evaluable with respect to the above parameters. Univariate analyses revealed a significant association between pathological axillary status and ER status (odds ratio (OR) 4.05, 95% confidence interval (95%CI) 2.81-5.83), PR status (OR 3.07, 95%CI 2.16-4.36), multifocality (OR 2.37, 95%CI 1.57-3.58), lymphovascular invasion (OR 8.61, 95%CI 5.12-14.46), detection of a SLN after NAT (OR .56, 95%CI .36-.87), detection method (IHC vs routine: OR .46, 95%CI .27-.78; IHC vs serial HE: OR .72, 95%CI .49-1.07; serial hematoxylin eosin (HE) vs routine: OR .639, 95%CI .39-1.04), clinical tumor size (OR 1.051, 95%CI 1.03-1.07) and pCR-status in the breast (ypT0 and ypTis vs others, OR .11, 95%CI .08-.17). A multivariate model was fitted including significant clinical parameters. Stepwise backward variable selection was carried out resulting in a model including ER status (OR 3.81, 95%CI 2.25-6.44), multifocality (OR 2.22, 95%CI 1.26-3.92), LVI (OR 9.16, 95%CI 4.68-17.90), detection of a SLN after NAT (OR .50, 95%CI .26-.95) and clinical tumor size (OR 1.03, 95%CI 1.01-1.06). In LOOCV, this model demonstrated an accuracy of 73% (sensitivity 73%, specificity 72%, PPV 75%, NPV 70%) using .5 as cut-off. Based on the performed ROC analysis an area under the curve (AUC) of 0.81 was calculated.

Conclusion: A model using ER status, multifocality, LVI, detection of a SLN after NAT and clinical tumor size was built to predict pathological axillary status (ypN+) with a high accuracy. If successfully validated based upon an independent dataset, this nomogram could allow advising patients for / against axillary surgery in case of clinical axillary conversion after NAT.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-07

Title: Are there patients with T1-T2, node-negative breast cancer who are high-risk for locoregional recurrence?

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Body: Background: Indications for post-mastectomy radiotherapy (PMRT) in T1-T2, node negative (N0) breast cancer patients with “high-risk” features are controversial based on lack of consensus as to what constitutes “high-risk”, and variable results of small retrospective studies. The EORTC 22922 and MA20 trials reporting improved 10-year disease-free survival with nodal irradiation included high-risk N0 patients but these patients were not analyzed separately and did not receive modern systemic therapy. We sought to evaluate long-term locoregional control in T1-T2N0 patients with high-risk features undergoing mastectomy in the contemporary era.

Methods: We retrospectively identified patients with T1-T2N0 breast cancer with ≥ 1 high-risk feature treated with mastectomy from 1/2006-12/2011. High-risk features were defined as age <40 years, multifocal/multicentric disease, lymphovascular invasion (LVI), medial or central tumor location, and high nuclear grade. The primary outcome of interest was rate of LRR.

Results: Among 672 patients meeting inclusion criteria, 187 (28%) had 1 risk factor: 21 (3%) were age <40 years, 132 (20%) were multifocal/multicentric, and 34 (5%) had LVI; 449 (67%) patients had ≥ 2 high-risk features, and 36 patients with unknown grade were excluded from risk analysis. PMRT was received by only 15 (2%) patients. Clinicopathologic characteristics of the 657 patients treated without PMRT are shown in Table 1.

Table 1: Clinicopathologic characteristics, n = 657

	Median (Range)	
Age, years	49 (24-89)	
Tumor size, cm	1.4 (<0.1-5.0)	
	n (%)	
Ductal histology	566 (86%)	
High nuclear grade*	266 (40%)	
LVI	232 (35%)	
Multifocal/multicentric	447 (68%)	
Medial/central tumor	226 (34%)	
Receptor status**		
ER+/HER2-	438 (67%)	
HER2+	123 (19%)	
ER-/HER2-	70 (11%)	
	n (%)	Rate of LRR
# of risk factors*		
1	183 (28%)	3.8%
2	265 (40%)	5.3%
3	143 (22%)	4.9%
4 or 5	32 (5%)	9.4%

*Unknown grade in 34 cases, excluded from risk analysis **Unknown receptor status in 26 cases

Sentinel node biopsy alone was performed in 98% of these patients. A median of 4 lymph nodes were retrieved (range 1-15). Adjuvant systemic therapy was received by 86% of patients. At median 5.6 years of follow-up, overall LRR rate was 4.7% (n = 31), with the majority (55%) of events involving the chest wall. Increasing tumor size was associated with LRR (HR 1.70, 95% CI

1.26–2.29, $p = 0.006$), while age, histology, grade, subtype, LVI, multifocality/multicentricity, and tumor location were not (all $p > 0.05$). Although rate of LRR increased from 3.8% to 9.4% with 1 vs. ≥ 4 high-risk features, a comparison of 1 vs. 2 vs. 3 vs. ≥ 4 risk factors was not significant by Kaplan-Meier estimation ($p = 0.54$).

Conclusions: A low LRR rate of 4.7% was seen in this large unselected cohort of T1-T2N0 cancers with "high-risk" features treated by mastectomy and systemic therapy without PMRT. While increasing tumor size was predictive, other features did not confer a higher risk of LRR either independently or together, and do not by themselves mandate the use of PMRT in this population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-08

Title: Preclinical validation of a new tumor imaging agent targeting $\alpha_v\beta_3$ to detect breast tumor using NIR-light imaging

Beurrier F, Dutour A, Chen Y, Froc E, Gayet P, Guillermet S, Rizo P, Chopin N, Faure C, Dammaco MA A, Klinger S, Ferraioli D, Garin G and Treilleux I. Centre Leon Berard, Lyon, Rhone-Alpes, France and Fluoptics, Grenoble, Isere, France.

Body: Surgery is still an essential step for breast cancer treatment. Complete tumor removal during surgery relies on the surgeon's ability to differentiate tumor from normal tissue. To date, no intra-operative method is available and reliable enough to help the surgeon delineate precisely tumor extension in the adjacent normal tissue. Thus, surgeons rely solely on pre-operative imaging techniques to delineate tumor margins. Recent advances made in the field of non-invasive imaging and vectorized nano-size particles have revealed a remarkable potential for improved tumor-margin detection. Angiostamp™ is a new fluorescent agent for intra-operative imaging. This system provides strong binding on $\alpha_v\beta_3$ integrin-rich tumor neoangiogenesis. Our general hypothesis is that targeting $\alpha_v\beta_3$ integrin-rich breast carcinoma neoangiogenesis should provide improved delivery of diagnostic compounds and assist surgeons intra-operatively using near-infrared imaging techniques.

We conducted preclinical studies to evaluate the distribution of Angiostamp™ in vivo in murine breast tumor model and to determine the expression of $\alpha_v\beta_3$ integrin on human breast specimens.

The capacity of Angiostamp™ to target $\alpha_v\beta_3$ integrin was assessed in 4T1 breast tumor model. After tumor implantation, mice were injected with Angiostamp™ or a saline solution. Detection of the tumors was performed at different timepoints (early, progressive and established tumors, N= 24 mice/timepoint) using near infrared (NIR) imaging system (Fluobeam™) to detect fluorescence in tumor tissue.

In parallel, the expression of $\alpha_v\beta_3$ integrins in normal breast tissue, benign lesions (N=20) and various tumor subtypes (N=120) was assessed by immunohistochemistry.

In mice injected with Angiostamp™, fluorescence was observed only within the tumors with low background. Angiostamp™ labeled tumors at all timepoint. No false negative fluorescence was found as confirmed by histopathological analyses.

In humans, the $\alpha_v\beta_3$ integrin are expressed in normal breast epithelial cells and benign metaplastic or proliferative epithelial lesions. However, due to the density of carcinomatous cells, the level of expression was much higher in breast cancer: 94% of invasive ductal carcinomas and 100% of invasive lobular carcinomas had a membranous staining (moderate to high intensity). Expression in breast cancer was not restricted to tumor subtypes neither hormone receptor expression nor SBR grade.

Based on this preclinical demonstration, Angiostamp™ could allow a better intra-operative detection of tumor bed in breast cancer, increasing the efficiency of surgical procedure especially for infraclinical disease and decreasing the rate of second surgery. Preclinical development is ongoing and the first clinical trial is expected in 2017.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-09

Title: Prediction of underestimation associated with flat epithelial atypia, atypical ductal hyperplasia and atypical lobular hyperplasia by needle biopsy: Experience in an Argentine breast unit

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Body: INTRODUCTION: Flat Epithelial Atypia (FEA), Atypical Ductal Hyperplasia (ADH), and Atypical Lobular Hyperplasia (ALH) are well known non-obligate precursors of breast malignancy. Management of FEA, ADH and ALH after image-guided needle biopsy (NB) still remains controversial. The aim of this study was to evaluate the rate of malignancy underestimation associated to FEA, ADH or ALH diagnosis by image-guided NB, either core needle biopsies (CNB) or vacuum assisted breast biopsy (VABB). As a secondary objective, we attempted to identify clinical characteristics and imaging features associated with malignancy.

MATERIAL AND METHOD: We retrospectively reviewed all consecutive image-guided NB performed between January 2006 and April 2016 that resulted in a diagnosis of FEA, ADH or ALH which were subsequently submitted to surgical biopsy. All pathological reports were available. We compared the pathology specimens obtained from biopsy to those obtained from surgery. The rate of underestimation was calculated by means of specific mathematical equations.

RESULTS: Among 4715 patients who underwent image guided NB, 174 (3.7%) resulted in diagnosis of FEA, ADH and ALH. 49 patients were excluded because of loss in follow up. The diagnosis in the remaining 126 cases, were 45 (36%) FEA, 66 (51%) ADH, 9 (7%) ALH and 8 (6%) combined diagnoses. Of these, 16 (13%) were ultrasound-guided CNB procedures and 110 (87%) stereotactic VABBs. In thirty-six cases (28,3%) the diagnosis was upgraded to malignancy. For CNB specimens, we found underestimation in 10 of 16 procedures (62.5%) and at VABB there were 26 cases of underestimation out of a total of 110 biopsies (23%). Univariate analysis indicated that use of CNB ($p=0.001$), residual calcifications ($p=0.008$), and presence of ALH in NB specimens ($p=0.08$) were independent predictors of underestimation. The presence of a mass on ultrasound was more likely to be associated with malignancy, as 5 out of 10 lumps (50%) resulted in lesion upgrade. Neither age at diagnosis nor hormonal status were significant predictors for malignancy underestimation. The final pathological results of the underestimated NBs, distributed by lesion type were as follows: For FEA, we found 7 low grade DCIS, 1 high grade DCIS and 1 invasive tubular carcinoma. Among 20 cases of ADH, we encountered 15 low grade DCIS, 1 invasive tubular carcinoma, and 4 NST ductal carcinoma. In 5 cases originally diagnosed as ALH we upgraded to LCIS, we found 1 invasive lobular carcinoma, and 1 invasive ductal carcinoma. Finally, both of the combined lesions resulted in upgrade to invasive ductal carcinomas.

CONCLUSION: In our experience, we found that the diagnostic underestimation rate when using CNB is approximately three times than for VABB. Residual microcalcifications and presence of ALH on NB were found to be independent predictors of underestimation. In our country, surgical excision of FEA, ADH and ALH remains the standard of care. Further research is needed in order to establish which patients with atypical findings on initial biopsy will achieve benefit from surgery, and which might be suitable candidates for surveillance.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-10

Title: A comparison of the micro-impulse radar SAVI SCOUT to the radioactive I125 seed in localization of non-palpable breast cancer for breast conserving therapy

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Body: Breast conservation and primary radiation (BCT) is a widely used treatment for early stage breast cancer patients. Studies report a 20-40 percent re-excision rate to obtain clear margins. Current localization practices include needle, radioactive seed and intraoperative U/S. Seed localization has been found to decrease tissue volume excision and improve patient satisfaction. However, radioactive seed programs are difficult to implement due to cumbersome regulations by the Nuclear Regulatory Commission (NRC). SAVI SCOUT® is a new technology cleared by the FDA for tumor localization. This device utilizes non-radioactive, micro-impulse radar (radar) to provide surgical guidance. The aim of this study was to evaluate the SCOUT® and determine its equivalence to seed localization by comparing re-excision rates and specimen volume.

70 patients with clinical stage 0, I, or II breast cancer who were treated with BCT were included in this IRB approved review. 35 patients were compared using the SCOUT radar localization technique with 35 patients using the radioactive iodine 125 seed localization technique. All patients received a wide segmental resection. The tissue was oriented and assessed clinically (visualization and palpation) and radiographically (Kubtec's XPERT 40 Digital Specimen Radiography System) in the operating room. Additional margins were excised if deemed to be suspicious by the surgeon (unless the anterior margin was skin or the posterior margin was the pectoral muscle fascia). Final margin status for both groups was compared. A positive margin was any margin with tumor on ink. The total volume of the excised specimen plus additional margins was recorded by the pathologist. In all 70 patients, the targeted lesions, seed and/or reflectors were successfully removed. There were 420 margins assessed (6 for each specimen), using the additional margins excised as the final margin for evaluation of tumor on ink. Of the 210 final margins in the specimens excised using the seed, 5 margins (2.38%) in 4 patients were positive. 5 margins (2.38%) were also positive in 4 patients using the SCOUT. Nine patients in total returned to the operating room for re-excision. [One re-excision was performed as physician preference for close (<2mm) margins for DCIS.] 5/9 patients requiring re-excision were found to have residual disease. A total of 119 additional margins were excised from 51 patients at the initial operation. 68 margins from 26 patients (SCOUT) and 51 margins from 25 patients (seed). Of the 119, 5 margins were found to be positive. 3 margins 4.41% (3/68) in the SCOUT group and 2 margins 3.92% (2/51) in the seed group. The average volume resected from the SCOUT averaged 81.28 cm³ while the volume of the seed averaged 100.39 cm³ (p-value 0.209).

The use of SCOUT for non-palpable tumor localization was equivalent to seed localization when comparing margin re-excision rate and tissue volumes. We conclude that SCOUT is an excellent alternative in breast cancer localization and can be easily implemented in most hospitals for breast conservation therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-11

Title: Utility of LigaSure™ vessel-sealing device in axillary dissection for breast cancer surgery: A randomized single center study

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Body: Introduction

Axillary lymph node dissection is standard therapy for patients with positive-node breast cancer, and can be performed with an electrocautery scalpel and suture ligation in most cases. However, knot slipping can occur during suture ligation and this can spread thermal damage to peripheral tissues. The LigaSure™ Small Jaw vessel-sealing system was developed as an alternative to suture ligatures, staplers, and other energy-dependent devices for sealing blood and lymphatic vessels, but its use in axillary dissection for breast cancer is limited. We prospectively compared the duration until drain removal after surgery, total lymph fluid drainage volume, intraoperative blood loss, and incidence of complications after axillary dissections, between this device and conventional methods.

Methods

This prospective randomized study was conducted at the Department of Breast and Endocrine Surgery at Tokai University School of Medicine, Kanagawa, Japan, between October 2011 and March 2015. Major eligibility criteria included (1) pathologically confirmed breast cancer diagnosis, (2) age ≥ 20 and ≤ 80 years, and (3) a signed informed consent form. The primary endpoint was duration until drain removal after surgery. The secondary endpoints were total lymph fluid drainage volume, intraoperative blood loss, and incidence of postoperative surgical complications. We defined the criterion for drain removal as a lymph fluid drainage volume of < 40 mL/day for two consecutive days. The target accrual was 100 patients, with a two-sided error rate of 5%, and 90% power. The assumed duration until drain removal after surgery was 7.2 days in the control group (conventional use of an electrocautery scalpel and suture ligation) and 5.8 days in the study group (use of the LigaSure™ Small Jaw). This clinical study was approved by the Institutional Review Board of the Tokai University School of Medicine and is registered with UMIN (No. 000013034).

Results

Initially, 100 patients were assigned as eligible; however, two patients were later excluded because of the exclusion criteria. Of 98 patients, 49 were randomized to the study group, and 49 to the control group. The mean duration until drain removal after surgery was 5.2 days in the study group and 5.0 days in the control group ($p=0.573$). The mean total lymph fluid drainage volumes were 260.3 and 233.5 mL ($p=0.502$), and the mean intraoperative blood loss volumes were 17.8 and 18.0 mL ($p=0.949$), for the study and control groups, respectively. No significant differences were found between the two groups regarding drain removal duration, total drainage volume, and intraoperative blood loss volume. Both groups had low incidence rates of postoperative hematoma, wound infection, lymphedema, and pain, and had similar incidence rates of seroma formation after drain removal.

Conclusion

Our study results indicated that the use of the LigaSure™ Small Jaw in axillary dissection for breast cancer was as safe as conventional methods. However, using the LigaSure™ Small Jaw did not improve surgical outcomes such as duration until drain removal and total lymph fluid drainage volume compared with conventional methods.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-12

Title: The concept of breast contour preserving surgery as parameter in breast cancer surgery

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Body: Background

Breast cancer treatment is a multimodality effort to optimize outcome as well as to achieve the optimal cosmetic result. Various treatment strategies can be used for the latter goal: upfront breast conserving surgery (BCS), BCS after neoadjuvant therapy (NAC), and ablative surgery combined with immediate breast reconstruction. The rate of BCS is frequently used as a quality and trend indicator. The aim of the present study was to analyse the combined efforts expressed as the rate of breast contour preserving procedures (BCPP) and compare it to the rate of BCS.

Material and methods

All invasive M0 female breast cancer patients diagnosed and operated in one of the 89 hospitals in the Netherlands between January 2011 – December 2015 were selected from the national NABON Breast Cancer Audit. BCPP (defined as 'primary BCS', 'BCS after NAC', or 'ablative surgery combined with an immediate reconstruction') was calculated for the years of diagnosis, age groups (<30, 30-39, 40-49, 50-59, 60-69 and ≥ 70 years) and the individual hospitals.

Results

A total of 61,309 patients were identified. The rate of upfront BCS remained stable during the study period (52%), while the BCPP rate increased over the years (63% to 71%) due to an equal increase in the proportions of patients receiving NAC with BCS and undergoing ablative surgery with immediate breast reconstruction. While upfront BCS (with and without NAC) rates increased with age (30% in patients aged <30 years to 67% in patients aged 60-69), the rate of BCPP was more or less stable in these age groups, as the rate of ablative surgery with immediate reconstruction showed an inverse relationship with age, decreasing from 44% in patients <30 years to 1% in patients ≥70 years of age.

The rate of BCS varied between hospitals in the Netherlands: 37% to 77%. Although BCPP is more often performed compared to BCS, the variation between hospitals remained (47% to 88%)

Table. Percentage of patients treated with Breast Conserving Surgery (BCS), BCS after neo-adjuvant therapy and immediate breast reconstruction with ablative surgery resulting in total percentages of Breast Contour Preserving Procedures (BCPP).

	BCS	BCS Neo-adjuvant +	Ablative surgery IBR +	BCPP
Year of Diagnosis				
2011	53%	4%	6%	63%
2012	53%	4%	7%	64%
2013	52%	6%	8%	67%
2014	52%	8%	9%	69%
2015	52%	9%	11%	71%
Age Group				
<30	17%	13%	44%	73%
30-39	27%	14%	26%	67%
40-49	38%	12%	17%	68%
50-59	56%	7%	11%	75%
60-69	63%	5%	5%	72%

≥ 70	49%	2%	1%	52%
Hospitals				
Mean	52%	6%	8%	67%
Min	34%	0%	0%	47%
Max	66%	26%	28%	88%

BCS: Breast Conserving Surgery; IBR: Immediate Breast Reconstruction; BCPP: Breast Contour Preserving Procedures

Conclusions

While the rate of BCS remained stable over recent years, the rate of BCPP has increased significantly. Including immediate reconstruction into the BCPP rate annihilates observed age-dependent differences of the BCS-rates, while institutional differences remained. All in all, combining different treatment strategies into one parameter (BCPP) provides a more appropriate measure of maintaining the breast contour than BCS alone.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-13

Title: Breast-conserving surgery plus hormone therapy without irradiation in elderly women with early breast cancer

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Body: Background

The meta-analysis of the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) revealed that whole-breast irradiation after breast conserving surgery (BCS) decreased ipsilateral breast tumor recurrence (IBTR) and breast cancer-related death. However, elderly patients can find daily hospital attendance difficult, and their IBTR risk is low. A randomized control trial was performed in women aged ≥ 70 years with hormone-positive breast cancer to investigate the usefulness of breast-conserving therapy without irradiation (Hughes et al. N Engl J Med 2004). Since September 2001, we have offered BCS plus hormone therapy without irradiation in patients who satisfy the following criteria: age ≥ 60 years; pathologically node negative, hormone-positive breast cancer; a negative surgical margin; and no lymphovascular invasion. We assessed prognosis in patients who chose this option.

Patients and methods

Between September 2001 and December 2014, 219 patients met the inclusion criteria; 90 and 129 patients underwent BCS plus hormone therapy with or without irradiation, respectively. The cumulative IBTR incidence and overall survival (OS) for the groups was evaluated. A negative surgical margin was defined as a margin of ≥ 5 mm.

Results

The median ages at operation were 73 years (range, 60–88 years) and 65 years (range, 60–80 years) for the without and with irradiation groups, respectively ($p < 0.001$). There were no significant differences in tumor size, lymph node metastasis, or adjuvant therapy between the groups. The median follow-up duration is presently 4.6 years. IBTR was observed in 5 (3.9%) and 1 (1.1%) patient(s) in the without and with irradiation groups, respectively ($p = 0.192$). The 5-year IBTR cumulative incidences were 0.9% and 2.2%, and the 10-year were 6.7% and 2.2%, for the without and with irradiation groups, respectively ($p = 0.390$). The 5-year OS rates were 93.8% and 98.5%, and the 10-year OS rates were 89.7% and 94.0 for the without and with irradiation groups, respectively ($p = 0.205$).

Conclusion

BCS plus hormone therapy without irradiation in elderly patients is an appropriate option.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-14

Title: Clinicopathologic factors related to surgical margin involvement, reoperation, and residual breast cancer in primary operable breast cancer - An analysis of 2050 patients

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Body: Background: Positive or involved margins resulted in higher rates of local recurrence in breast cancer patients and often needs further operations. Identification of patients at increased risk for positive surgical margin may enhance clinical pre-operative decision-making.

Methods: A retrospective study with 2050 primary operable breast patients were enrolled in our current margin analysis study, and factors associated with positive surgical margin, re-excision, and residual cancer detection in the re-excision specimen were analyzed.

Results: Among them, 151 (7.4%) patients were found to have positive surgical margin involvement. The positive surgical margin involved rate was 11.3% (118/1042) in breast conserving surgery (BCS) patients, and 3.3% (33/1008) in mastectomy group (P<0.001). In multivariate analysis, the positive surgical margin involvement in BCS patients was associated with lower body surface area (BSA), larger tumor size (OR=1.377, CI=0.147~0.498), and pathologic multifocal (OR=3.766, CI=0.639~1.973). MRI use was associated with decrease margin involved rate in BCS patients (OR=0.530, CI=-1.102 ~-0.172). Among the 33 margin involved mastectomy patients, only 3(9.1%) received further excision. Among the 118 BCS patients with surgical margin involvement, 83(70.3%) received further re-excision. Patients with younger age, or DCIS (OR=2.165, CI=0.225~ 1.464) histologic subtypes were factors related to re-operation. Combined with MRI for pre-operative evaluation was associated with decreased re-operation (OR=0.302, CI=-2.129 ~ -0.317)

Table 1 Univariate and multivariate analysis of margin involvement in breast conserving surgery patients

	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	p	Odds ratio	95% CI	p
BSA	0.143	-3.365~-0.560	0.007			
BMI	0.919	-0.141~-0.003	0.032	0.866	-0.214~0.078	<0.001
Biopsy method(CNB)	1.054	-0.227~0.309	0.702			
Gross tumor size	1.270	0.073~0.407	0.004	1.377	0.147~0.498	<0.001
Distance to skin	0.551	-1.090~-0.148	0.013			
Pathological multifocal (Yes)	3.285	0.587~1.754	<0.001	3.766	0.639~1.973	<0.001
MRI (Yes)	0.486	-1.118~0.335	<0.001	0.530	-1.102~-0.172	0.007
Stage	0.854	-0.343~0.016	0.087			

. Among the 83 BCS patients with positive surgical margin and undergone further surgical excision, 44(53%) was found to have residual tumor in the re-excision specimens. Compared with no residual tumor in the second operation group, no difference in tumor size, lymph node status, pathologic multifocal, or MRI use were found. The only difference was that DCIS (71%, 22/31) histologic subtype was associated with higher residual tumor than other (42.3%, 22/52) type of breast cancer (P=0.021).

Conclusions: Lower BSA, larger tumor size, pathologic multifocal were associated with increased risk for positive surgical margin involvement. Combined pre-operative evaluation with MRI was associated with decrease surgical margin involvement and re-operation in BCS patients. Patients with DCIS had positive surgical margin involvement was associated with increased risk for re-operation and residual cancer found at re-excision.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-15

Title: An update on trends in nipple-sparing mastectomy incision locations: A systematic review of the literature with pooled analysis

Santos PJF F, Daar DA A, Mowlds DS S, Wirth GA A and Lane KT T. University of California Irvine School of Medicine, Irvine, CA.

Body: Introduction: The incidence of nipple-sparing mastectomy (NSM) has greatly increased, owing to refinements in technique, increased surgeon comfort and the superior aesthetic outcome afforded by retention of the nipple-areola complex (NAC).¹ The indications for nipple-sparing mastectomy continue to broaden as the oncologic safety of nipple preservation is elucidated.² Incision location varies widely among surgeons and likely contributes to the complication profile associated with these procedures. While small studies have looked at complications associated with NSM,³ the preferred incision location has not been determined.

Methods: A systematic review was performed according to the Preferred Reporting Items for Systematic Review (PRISMA) guidelines. Search terms “nipple-sparing,” “nipple-areola sparing,” or “total skin-sparing” were used in PubMed and MEDLINE databases from January 2013 to October 2015. Studies were included if the mastectomy incision type was described and accompanied by the overall and/or respective complication profile. A pooled analysis was completed for the overall NAC necrosis rate as well as for necrosis by incision type.

Results: Of the 162 studies identified, 32 studies met the inclusion criteria, and a total of 4,986 nipple-sparing mastectomies were available for analysis. Incision types were divided into 6 categories: inframammary (2,030 NSMs, 40.7%), radial (1,247 NSMs, 25%), periareolar (922 NSMs, 18.5%), endoscopic (444 NSMs, 8.9%), mastopexy/previous scar (271 NSMs, 5.4%), and other (72 NSMs, 1.4%). Thirty different incision variations were used, in total. Among the 26 studies reporting overall NAC necrosis rates (3,831 NSMs), the pooled NAC necrosis rate was 9.3%. Seventeen studies reported their rates of necrosis by incision location (1,736 mastectomies): inframammary, 11.6%; radial, 13.8%; periareolar, 18.2%; endoscopic, 17.4%; mastopexy/previous scar, 5.7%; and other, 0.0%.

Conclusion: The inframammary incision has become the preferred NSM incision as it minimizes disruption of the blood supply to the NAC as evidenced by its superior complication profile. Many studies fail to report their complications as they relate to incision location. Consistent reporting in the literature is warranted as a means of further elucidating the short and long-term complication profiles associated with NSM incision location.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-16

Title: 200 experiences of oncoplastic surgery with the omental flap reconstruction

Abe N, Sagawa N, Unesoko M and Zaha H. Nakagami Hospital, Okinawa, Japan.

Body: Background: There are several small case series about the omental flap for breast reconstructions. However, the long-term oncological safety and the clinical benefits remain uncertain, especially the flap is used as a part of oncoplastic breast surgery. We retrospectively studied 200 patients who underwent oncoplastic breast surgery using the laparoscopically harvested omental flap.

Methods: A retrospective chart review of 200 patients who underwent oncoplastic surgery using the omental flap at our institution from April 2002 to March 2016 was performed. All the omental flaps were laparoscopically harvested, and mainly used as a partial reconstruction immediate after breast-conserving surgery (BCS). Short- and long-term laparoscopy-associated complications, local recurrences and cosmetic outcomes were evaluated.

Results: Although 46 cases of nipple-sparing mastectomy or skin-sparing mastectomy were included, most of patients underwent partial breast reconstruction after BCS. The successful rate of laparoscopically harvesting of the omental flap was 99.5% (199 out of 200). The rate of complications was 12.0%. Laparoscopy-associated complications occurred in 4 cases (2.0%). The rate of positive margin was 6.5%. Local recurrence occurred in two cases (1.0%) during medial follow-up period of 78 months. In 24 patients (12.0%), the volume of the flap was insufficient. When applied to nipple-sparing mastectomy or skin-sparing mastectomy, volume lack occurred in 32.6% of patients. Cosmetic outcome was mostly satisfactory. Approximately 80% of patients scored good or excellent evaluated with 4 point scale by 3 health professions. The size of the reconstructed breast basically did not change even after radiation therapy. Donor-site scars were almost negligible just like those of laparoscopic cholecystectomy.

Conclusions: The laparoscopically harvested omental flap has minimal donor-site morbidity and deformity. The long-term oncological safety is also promising. Although there is a limit of adaptable volume, the omental flap is an attractive option in partial breast reconstruction after BCS.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-17

Title: A multi-center prospective study of radiofrequency ablation therapy for small breast carcinomas

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Body: Background: As the management of breast carcinoma evolves toward less invasive treatments, the next step is the possibility of removing the primary tumor without surgery. The most promising noninvasive ablation technique is radiofrequency ablation (RFA), which can effectively kill tumor cells with a low complication rate. Our preliminary studies of RFA followed by standard surgical resection have indicated that this technique is effective for surgical ablation of small (≤ 2 cm) breast tumors without extensive intraductal components (EIC).

Methods: To determine if RFA is oncologically and cosmetically appropriate for the local treatment of primary breast carcinoma, this multi-center prospective study used RFA as the sole local treatment of breast tumors ≤ 1.5 cm in size on ultrasound and MRI. Exclusion criteria include receiving of preoperative chemotherapy, or the presence of invasive lobular carcinoma or invasive ductal carcinoma with suspicious EIC. After confirmation that the standard baseline core biopsy for diagnosis and measurement of tumors markers (ER, PgR, HER-2/neu expression and the presence of the Ki-67 proliferative marker) have been obtained, consent will be obtained and the patient scheduled RFA. All patients received adjuvant radiation therapy. The use and choice of systemic therapy will be based on the information from the baseline core biopsy. The first primary endpoints of this study is successful tumor ablation, as evidenced by negative findings on vacuum-assisted or core biopsies and imaging studies after RFA. The second primary endpoints is the incidence of procedure related adverse events. Forty patients with small tumors that are clearly identifiable and measurable by ultrasound and MRI were enrolled. The response to ablation was evaluated with both vacuum-assisted or core biopsies and imaging studies every 3 months during the first year. The long-term outcomes were assessed using quality of life measurement scales and imaging studies every 6 months thereafter through year 5.

Results: Of the 58 patients who participated in this study, 55 completed the protocol. In 48 of the 55 (87%) treated patients, successful tumor ablation, as determined by negative findings on vacuum-assisted or core biopsies and imaging studies, was confirmed. The remaining 7 patients with biopsies positive for residual tumor underwent surgical resection. There were no local or distant recurrences in treated 55 patients with a median follow up of 47 (range 36-73) months.

Conclusions: RFA can be safely used alone in patients with small breast tumors, provided that local tumor control must be regularly assessed by image-guided vacuum-assisted or core biopsies after ablation. RFA has several potential advantages over lumpectomy for the treatment of early stage breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-18

Title: Technical skill of surgical residents may affect margin status of breast conserving operations

Komenaka IK K, Djenic B, Hsu C-H, Nodora J, Winton L, Bouton M and Martinez ME. Marciopa Medical Center; University of Arizona and University of California San Diego.

Body: Background: Quality of patient care and surgical outcomes have come under increased scrutiny. Numerous other studies have examined outcomes at teaching hospitals compared to non-teaching hospitals. The most important factor to determine early success of breast conservation is the attainment of pathologically negative margins. Few studies, however, have examined the effect of teaching residents on margin status. The current study was performed to evaluate the effect teaching residents on margin status after lumpectomy.

Methods: Retrospective review of all patients from July 2006-June 2015 was evaluated. A resident was usually considered the primary surgeon. If the resident was unavailable or unprepared to operate, the attending surgeon was the primary surgeon. As part of the routine evaluation of surgical residents, technical ability was classified as satisfactory or unsatisfactory for level of training. All evaluations of the technical ability of the residents were completed prior to the collection of the current data. The effect of surgical residents' participation and their technical ability to perform lumpectomy was evaluated to determine if there was an effect on margin status. Logistic regression analysis was performed to adjust for clinical variables known to affect margin status.

Results: Of 292 patients, 15% of patients had positive margins. The attending surgeon has positive margin rate of 10.7% vs 16% for Residents ($p = 0.32$). When technical skill evaluation was included, Residents with unsatisfactory technical skills had positive margin rate of 27% compared to 10.2% for residents with satisfactory skills ($p = 0.002$). In multivariate logistic regression analysis, operating surgeon remained significantly associated with positive margins. Operations done by residents with satisfactory technical skills or attending surgeon were less likely to have positive margins than those done by residents with unsatisfactory technical skills (OR 0.39, 95% CI 0.22-0.86; $p = 0.03$). Patients who underwent preoperative chemotherapy were also less like to have positive margins (OR 0.40, 95% CI 0.18-0.91); $p = 0.04$). In patients with at least 2 years of followup (mean follow up of 48 months) breast cancer specific survival was 94% and 2% had local recurrences as a first event.

Conclusions: Technically ability of residents may affect margin status after lumpectomy. With the increased use of surgical outcomes to measure quality of care in medicine, the importance of teaching surgical residents needs to be considered in future quality of care evaluation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-19

Title: Isolated sternal metastases: The place of surgical resection

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Body: Background: Solitary sternal metastases from breast cancer are found in approximately 1.9 to 2.5 % of all advanced breast cancer cases. According to the latest AJCC classification, they are still considered as stage IV disease, but their prognosis is better in the absence of other foci of metastatic disease. Their treatment still remains controversial.

Material and methods: This is a monocentric retrospective study performed in our breast clinic and approved by our ethics committee. Twelve patients were included in this study between 2010 and 2015; 11 had a metachronous solitary sternal metastasis and 1 had a synchronous metastatic sternal lesion. Complete restaging was negative in all patients for other metastatic lesions. The extent of resection (different parts of the sternum and frequently also adjacent rib cartilages) necessary to obtain free margins was estimated preoperatively on MRI images focused with adequate sequences. All the patients underwent a large sternal resection and a chest wall reconstruction integrated in a multimodal approach.

Characteristics of the patients and of the tumors were studied.

The major outcomes studied were disease-free (DFS) and overall survival (OS).

Results: The mean interval between the initial diagnosis of breast cancer and the discovery of sternal metastasis was 115 months. After surgical resection, free margins were obtained in 10 patients. No post-operative complications were observed except for persistent thoracic pain in one patient with a medical history of chronic pain. Excellent cosmetic and functional outcomes were obtained without significant impairment of respiratory function. Ten patients received chemotherapy pre- or postoperatively. All the tumors expressed ER and /or PgR receptors, and endocrine therapy was administered in all patients. Mean duration of follow-up was 25 months (9-51 months). Three patients presented distant recurrences: 2 liver lesions and 1 cervical nodal recurrence with pericardic effusion. Out of these 3 patients, 1 died after 51 months. Currently the DFS of this small study is 75 % and the OS is 91.6 %.

Conclusion: Treatment of isolated sternal metastases of breast cancer must be based on a multidisciplinary strategy. Sternectomy and multilayered chest wall reconstruction (with different types of meshes and flaps) could be a curative approach in highly selected patients with no other metastatic lesions. In this group of patients, the good prognosis observed could be due to a different mechanism of dissemination, based on lymphatic rather than hematogenic diffusion. Longer follow-up and prospective studies are needed to confirm these encouraging results.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-20

Title: Use of a 3-D bioabsorbable tissue marker in 61 patients over two years

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Body: Background: Breast conservation surgery (BCS) may preserve the breast but many women have less than optimal cosmetic outcomes. Increasingly, this has been addressed by the use of oncoplastic reconstruction. Now, targeting radiation treatment for boost or partial breast irradiation (PBI) using the seroma has become more difficult due to the adjacent tissue rearrangement and resultant “benign” seromas. Since the radiation dose generated increases by the third power of the radius (r^3), there is a need to accurately focus radiation therapy to avoid chronic radiation side effects. We have used a 3-dimensional bioabsorbable tissue marker to accurately target the tumor bed while excluding inadvertent seromas caused by oncoplastic procedures. We report on tissue marker implantation on 61 consecutive breast conservation patients in regards to targeting, impact on cosmesis, imaging followup, ease of re-excision, as well as side effects and patient satisfaction.

METHODS: Consecutive lumpectomy patients who were candidates for targeted radiation therapy were considered for implantation with the 3-D bioabsorbable marker from May 2014 to June 2016. The tissue marker has a fixed array of 6 titanium clips and was sutured to the site of the excised breast cancer during lumpectomy. The framework of the implant resorbs slowly over time, while the clips remain permanently. All patients had oncoplastic reconstruction with total implant coverage. The marker was utilized for boost or partial breast irradiation (PBI) planning or treatment targeting. Data includes patient demographics, breast size, tumor characteristics, surgical and radiotherapy techniques, follow-up imaging, cosmesis and patient satisfaction.

Results: Data on 61 patients with median follow-up 12.7 months was analyzed (range 1.5–25.5). Median age was 62.4 years (range 33-74), 5 of women were postmenopausal and 15% had comorbidities. Cancer histology was in-situ (13%), invasive ductal (84%), invasive lobular (3%) of sizes T0 (13%), T1 (59%), T2 (25%), T3-4 (3%). Laterality and tumor location within the breast were typical. Re-excisions occurred in 11% of patients. No infections occurred in the postoperative period. One infection occurred with chemotherapy and another with repeated aspirations of oncoplastic area. No device was removed for infection, misplacement or patient-generated concerns. No cancer recurrences have been reported. Size of device used reflected size of the tumor; 2X2cm (44%), 2X3cm (34%) and 3X3cm (20%). The device was utilized by radiation oncologists for boost or PBI planning and treatment. Data on ease of setup and boost planning is being collected. Mammography at one year demonstrated marker clips coalescing as the bioabsorbable device dissolves. Evaluation of cosmetic appearance has shown good to excellent cosmesis as judged separately by both physicians and patients (92% and 94%).

Conclusions: Initial experience with 61 patients implanted with a novel 3-D absorbable device prospectively followed for an average of 12 months can be used in an array of breast cancer patients without device specific morbidity. Good to excellent cosmesis may be related to the addition of volume to the lumpectomy bed not seen with rearrangement of existing tissues.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-21

Title: Primary surgery in inflammatory breast cancer

Muzaffar M, Vohra N and Wong J. East Carolina University/Brody School of Medicine, Greenville, NC.

Body: Background: Inflammatory Breast Cancer (IBC) is a rare but very aggressive variant of breast cancer accounting for around 1-5% of all breast cancer cases. Current standard of care is multimodality therapy with preoperative chemotherapy followed by modified radical mastectomy and adjuvant radiotherapy.

Method and Material: We searched the Surveillance Epidemiology and End results Registry to identify female patients diagnosed with Stage III IBC between 1988-2013. We performed descriptive and univariate analyses of the patients with IBC. Variables assessed included patient age, race/ethnicity, histologic subtype, tumor grade, hormone receptor status, surgery type.

Results: We identified 11604 women with IBC from SEER database diagnosed between 1988-2013. The median age was 57 yrs., 80 % (9273) of patients were white, 14.4 % (1667) blacks, and around 73% had nodal involvement. 32.4 % (3756) patients were diagnosed with IBC between 1988-2000, while 67.6 % (7848) between 2001-2013. We excluded patients with missing primary surgery information with 7586 patients remaining for analysis. The median age in this final cohort was 56yrs, 80.4 % (6097) patients were white, and 13.7 % (1044) were black. The predominant tumor type was invasive ductal carcinoma, majority of the patients had grade 3 tumor 59.4 % (4504), grade 2 in 23.2 % (1757), 47.1% were estrogen receptor (ER) positive, 43% were ER negative, 53% were progesterone receptor negative, and 7% had HER 2 positive tumor. In this cohort 6.4 % (487) patients underwent partial mastectomy and 93.6 % (7099) underwent mastectomy. In the cohort undergoing breast conservation, 15% were during 1988-2000 and 84.4% during years 2001-2013 (p value=0.06) -

Surgery in non-metastatic inflammatory Breast Cancer

Type of Surgery	1988-2000	2001-2013	p-value
Partial Mastectomy	76 (6.7%)	1140(93.3%)	0.06
Mastectomy	411 (6.9%)	5959(93.1%)	

. The median survival was 46 months among patients diagnosed between 1988-2000 and 61 months for 2001-2013 time period ($p < 0.0001$). The median survival in patients undergoing partial mastectomy was 42 months versus 48 months among patients undergoing mastectomy ($p = 0.0001$).

Discussion: IBC continues to be an aggressive breast cancer subtype, but survival has improved over last decade. The utility of partial mastectomy has increased in the last decade compared to 1988-2000. Mastectomy still remains the standard surgical option, but with more effective systemic therapeutic agents, better tumor responses are noted especially in Her 2 positive and triple negative IBC. Partial Mastectomy may be a viable option for patients achieving complete clinical response.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-22

Title: Should modified radical mastectomy be modified? A phase 1 study to evaluate infraradical mastectomy for invasive breast cancer

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Body: Background: Surgical procedures in breast cancer have become less aggressive. However, around one in three patients must undergo modified radical mastectomy (MRM). We evaluated if infraradical mastectomy (IFM), which preserves the skin and fat tissues surrounding the breast, is safe. IFM has the potential to preserve neck opening and by the way femininity of patients. Aesthetic results obtained after breast reconstruction are also expected to be improved.

Objective: The primary objective of this phase 1 trial is to evaluate the feasibility and safety of IFM.

Methods: Patients were recruited in two specialized breast clinics. We performed enucleation with a cold knife to the mammary gland after a water-assisted dissection of the periglandular space using a dedicated tool. In a second step we removed the peripheral skin and fat tissue which surrounded the gland to obtain a classical MRM. Fat tissue removed in the second step underwent a careful pathological examination (10 biopsies) in order to evaluate residual invasive or in situ breast cancer and atypical hyperplasia. We also evaluated which is the percentage of tissue and skin removed in the second step with regard to the global surgical specimen because that could be left in the future if the procedure is declared safe.

Results: A total of 35 patients (43 to 80 years old) were prospectively recruited from March 2015 to March 2016. The distribution of tumor type was: pTis 2.9%, pT1 45.7%, pT2 45.7% and pT3 5.7%. Pathological analysis of the periglandular tissue removed in the second step revealed: 0% invasive carcinoma, 1% focal ductal carcinoma in situ (DCIS), and 0% atypical hyperplasia. On average, the weight of an IFM was 37% lower compared to the weight of a MRM. Skin resection was reduced by 48% with IFM. No serious adverse event was observed.

Conclusions: Preliminary results are promising. Our phase 1 trial suggests that the procedure is safe concerning premalignant or malignant disease left after IFM. Moreover, a high quantity of skin and fat tissue is saved by IFM. We are now recruiting a second cohort of patients in order to confirm these results. In addition, we will also quantify the amount of mammary glands left around IFM compared to MRM by performing additional biopsies and quantitative evaluation by pathologists.

Presurgical selection criteria have been modified for the second cohort in order to avoid residual DCIS left after IFM. After this second cohort, we plan to begin a large prospective randomized phase 3 trial with long-term disease-free survival and aesthetic results as the primary endpoints.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-23

Title: Predicting residual disease in breast conservative surgery after neoadjuvant treatments in breast cancer patients using the margin index tool

Rubio IT T, Loinaz I and Esgueva-Colmenarejo AJ J. Hospital Universitario Vall d'Hebron, Barcelona, Spain.

Body: Background. The appropriate surgical margin after neoadjuvant treatment (NAT) is controversial. Margin index, a relationship of the margin obtained to the size of the tumor, is a reliable method for the prediction of residual disease after breast conservative surgery (BCS) in the adjuvant setting. Our aim was to apply the margin index to patients with BCS after NAT to predict residual disease.

Material and Methods. From a prospectively maintained database, patients with stage I - III with NAT who underwent BCS between July 2008 and December 2012 were included. Margin index was calculated as for the adjuvant setting: margin index = closest margin (mm)/tumor size (mm) × 100 considering the tumor size of the lumpectomy specimen. A receiver operating curve was created using the derived margin index and the presence or absence of residual disease in the re-excision specimen. Patients with pathologic complete response (pCR) (no invasive tumor) were excluded.

Results. Of the 206 patients, 63 (30.5%) had a pCR and were excluded. Of the 143 patients, 76 (53%) had an intraoperative re-excision depending on the surgeon or the intraoperative pathology report. Of the 76 patients, 23 (32%) had residual tumor in the intraoperative re-excision specimen. There were no statistically significant differences in residual disease regardless of who indicates intraoperative re-excision. (p= 0.56) Twenty-three patients (16%) underwent a second surgery for positive or close margins (< 2mm). Residual disease, whether infiltrating or in situ or both, was found in 10 (43%) patients. The overall c index for the receiver operating curve was 0.76. A margin index > 5 had a sensitivity of 80% and a specificity of 63%.

Conclusion. Rates of re-excision after NAT in BCS patients are similar to the adjuvant setting. Margin index may be a valuable tool for determine residual disease after NAT in patients undergoing BCS. Further validation of this index is warranted in a larger cohort of patients after NAT to confirm these results.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-24

Title: Non-liposuctive endoscopic axillary lymph node dissection in breast conservative surgery

Yang W, Zhang H and Wang H. Fudan University Affiliated Zhongshan Hospital, Shanghai, China.

Body: Objective

Limiting surgical invasiveness is the main theme of breast surgery. Endoscopic axillary lymph node dissection (EALND) has been introduced since 1993 but not widely applied due to limited benefits as well as the controversy of liposuction that needed to create the working space in axilla. Progress of energy instruments brings the opportunity to new attempts to further enhance cosmetic outcomes and diminish complication rates. In this study, we report the preliminary outcomes of non-liposuctive EALND (NEALND) in breast conservative surgery.

Patients and methods

From Nov 2013 till June 2016, 143 patients diagnosed with cN+ breast cancer were categorized into two separate groups, the conventional ALND(CALND, 71 patients) and NEALND group(72 patients).

Informed consents were obtained from all patients. Number of lymph nodes harvested, tumor size, intra-operative bleeding, drainage volume, pain score (10scale survey) were collected.

The breast symmetrical score (BSS) and patient satisfaction (4scale survey) were used as variables to evaluate cosmetic outcomes.

Results

There was no statistical difference in intra-operative bleeding(NEALND:31.33±20.22ml, CALND:38.00±18.21ml, P=0.31) and first postoperative 3 day axillary drainage volume(NEALND:62.30±32.42, CALND:69.67±35.44, P=0.11). A slightly increased in the number of harvested lymph nodes was observed in the NEALND group in comparison with CALND (NEALND: 17.34±6.55, CALND: 14.68±5.85, P=0.03), there was no statistical difference reported for distant metastasis among two groups(NEALND:2.68±3.59, CALND:2.55±1.31,P=0.54). The pain score (NEALND: 3.79±1.51, open: 4.41±0.96, P=0.04) meanwhile breast symmetrical score was significantly superior in the NEALND group in comparison with CALND (NEALND: 0.098±0.091, CALND: 0.25±0.14, P=0.001) . Patient satisfaction survey reported a slightly positive feedback(NEALND: 4.00±0.00, CALND:3.77±0.54, P=0.02). In the CALND group, slight to moderate skew of nipple position towards the axilla was observed in many cases, suggesting the side effect of axillary scar contracture. The average operation time for NEALND was about 90min in the first 20 cases, and then down to about 60min, so the learning curve is suggested as 20 cases. All the patients are under follow up with no recurrence to date

Conclusions

Efforts on minimizing surgical invasiveness are greatly developed in systemic treatment and non-surgical local control. However, surgery is the fastest and the most economical way to obtain pathological complete remission, and still has space to be completed. NEALND may be a feasible surgical alternative with minimal invasiveness, suitable to a category of patient demanding excellent cosmetic outcome. Evaluation of long-term survival, complication rates and cosmetic results are mandatory in order to further estimate the applicability of this type of surgical management in treatment of breast cancer.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-25

Title: Oncoplastic breast surgery is oncologically safe in locally advanced breast cancer after neoadjuvant chemotherapy, an Egyptian experience

Youssef M, Namour A, Youssef O and Morsi A. National Cancer Institute, Cairo University, Cairo, Egypt and Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom.

Body: Background:

Oncoplastic surgery (OPS) has emerged as a new approach for extending breast conserving surgery (BCS) possibilities, reducing mastectomy and re-excision rates, while avoiding breast deformities. OPS integrates plastic surgery techniques for immediate reshaping after wide excision for breast cancer. This techniques is emerging and our experience in Egypt has been gradually increasing. Our aim was to extend the applicability of OPS into more advanced tumours following neoadjuvant chemotherapy.

Method:

A prospective feasibility cohort study of OPS after neoadjuvant chemotherapy was carried at the national Cancer institute – Cairo University and included 40 patients. We aimed to look at long term oncologic safety and cosmetic outcomes.

The primary outcome was the local recurrence rate. Secondary outcomes included survival and margins obtained as well as cosmetic outcomes. Survival analysis was performed with Kaplan-Meier curves. Cosmetic outcomes were assessed using a modified Breast Q questionnaire (EORTC 10801).

Results:

40 patients were included in this study. All were diagnosed with locally advanced breast cancer between September 2012 and January 2015 at the National Cancer Institute – Cairo University. All were treated primarily with neoadjuvant chemotherapy (Anthracycline-based). The median age was 44.45 (Range 22- 65) years with median follow-up period of 42 (Range 24 – 60) months.

27.5% showed complete pathological response. 62.5% of patients had a level I OPS procedure, 10% had a level II procedure, and 27.5% had a volume replacement procedure with a Latissimus Dorsi flap.

The median resection margins with level I, level II, and volume replacement were 10 mm, 25 mm and 15 mm respectively. The difference in margins between level I and II was statistically significant ($p = 0.028$), so was the difference between the 3 types of procedures ($p = 0.035$).

Three patients (7.5%) had local recurrence and required mastectomy; at 11, 13 and 16 months respectively. One of those (2.5%) developed distant bone metastasis.

Cumulative disease-free survival (DFS) for the whole cohort was 90.2%. Overall survival (OS) was 100% as there was no mortality reported during the follow-up period. Cumulative disease-free survival for patients with level I surgery was 85.4% while for those with level II and volume replacement it was 100% with no statistically significant difference ($p = 0.2$) because of small number of events.

The cumulative disease-free survival when the median resection margin obtained was less than 20 mm was 86.3%, whereas when the median margin was equal or more than 20 mm, it was 100%. This difference was not statistically significant ($p = 0.2$). The cosmetic outcomes ranged between excellent result (70%), very good (15%), good (10%) and poor results (5%) on a very simplified scale that was used for the purpose of the study.

Discussion:

Oncoplastic breast surgery didn't compromise oncologic safety in the patients included in the study. The local recurrence rate, the DFS and OS were all within acceptable ranges .It even allowed wider margins of resection which could be associated with better oncologic outcomes. At the same time, it gave a better cosmetic outcome and therefore higher patient satisfaction.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-26

Title: Deep Infrared Imaging to identify venous impairment after breast cancer surgery

Belgrado J-P, Vandermeeren L, Vankerckhove S, Valsamis J-B, Feipel V, Rooze M, Moraine J-J, Hertens D, Carly B and Liebens F. CHU St-Pierre - Brussels Lymphology Clinic, Belgium; BEAMS, Belgium; Université Libre de Bruxelles, Belgium and CHU St-Pierre - ISALA Breast Clinic.

Body: INTRODUCTION

Breast cancer related lymphedema (BCRL) is commonly attributed to axillary lymph node dissection (ALND) and reduction of lymph flow. Impairment of the axillary vein seems to contribute also to BCRL, leading to a deep pitting edema of hand and forearm. When a patient with axillary vein impairment stands up, hand skin looks rapidly hyperaemic, due to a vasodilation of the capillaries. This vascular situation may result from removal of the fatty tissue containing lymph nodes, and disruption of the good emptying of the axillary vein. The orthostatic intermittent venous stenosis induces collateralizations which are derivative evidence of the axillary vein impairment. We highlight them using an original, cheap and easy procedure, based on a deep infrared imaging (DIRI) device.

MATERIAL AND METHODS

A total of 100 women were recruited, 50 BCRL patients and a control group composed of 50 healthy women. In all subjects, we performed visible light and DIRI pictures of the thorax, including neck, shoulders and upper arms. Images were mixed and screened by 3 blind operators. The operators screened for differences in thermograms, such as asymmetric and collateral trajects.

RESULTS

The DIRI coupled with our reading grid seems to be specific and sensitive enough to identify BCRL patients with asymmetric collateralization of the axillary vein.

CONCLUSION

DIRI and its reading grid seems to be a useful tool in daily clinical practice to evaluate the hemodynamic changes of the axillary vein in BCRL patients. This evaluation gives us more insight in the (future) development and eventual treatment of BCRL.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-27

Title: Early detection of secondary lymphedema after cancer treatments

Belgrado J-P, Vandermeeren L, Vankerckhove S, Valsamis J-B, Carly B, Hertens D, Jeanjot I, Beier B, Etbaz S, De Coster C and Liebens F. CHU St-Pierre - Brussels Lymphology Clinic, Belgium; BEAMS, Belgium and CHU St-Pierre - ISALA Breast Cancer Clinic, Belgium.

Body: Introduction

The overall incidence of secondary lymphedema in oncological surgery is estimated at 15,5%. Most of filtrated fluid returns to circulation by the lymphatic system. Reducing the lymphatic capacity transport, lymph node dissection procedures shift the fluid balance exchange, leading, in a time-delayed manner, to the onset of a lymphedema. All patients undergoing an adenectomy are at the risk to develop a lymphedema. Complaints of heaviness, paresthesia or power loss in the ipsilateral arm are precursor signs of secondary lymphedema which are not enough considered to start a decongestive treatment. Currently, a lymphedema is diagnosed after it has clinically developed.

In order to start earlier decongestive treatment or preventive treatment, sensitive detecting tools can be helpful. Actually no instrument or method has demonstrated the capability to detect accurately pre-clinical signs of secondary lymphedema. Near infrared fluorescence lymphatic imaging (NIRFLI) could be the answer to this need.

Our experience based on almost thousand NIRFLI exams on healthy and lymphedema patients highlights that the superficial lymphatic system is completely different in healthy limbs compared to lymphedematous limbs. NIRFLI exams in oncological patients who are apparently free of secondary lymphedema, after surgery, present typical abnormalities of the lymphatic network. This study aims to confirm the possibility to detect secondary lymphedema at a subclinical stage. Detection may be performed using NIRFLI that allows realizing mapping of the superficial lymphatic network at different times after surgery in order to follow the patient at risk to develop secondary lymphedema.

Method

Breast cancer patients are recruited before surgery and after informed consent. They undergo NIRFLI exam before surgery, and 10 days, 3 months, 6 months, 1 year and 2 years after surgery. Images and videos are recorded and compared to the previous one in order to detect any change in the architecture of the superficial lymphatic system. Anamnesis, clinical examination of the operated area, precise volumetry and deep infrared imaging are performed at each session. The study started in January 2015, it was approved by the local ethical committee of the CHU St-Pierre – Université libre de Bruxelles (CE AK/13-12-119/4322Bis; EudraCT number: 2013-004237-32;NCT02415725).

Results

To date, 30 patients have been recruited. One patient presents changes in her NIRFLI, and presents also signs of lymphedema, one year after the treatment. The other patients do not show any changes in their NIRFLI, nor preclinical or clinical signs of lymphedema. No side effects consecutive to the NIRFLI exams were noted.

Conclusion

After almost thousand NIRFLI in lymphedema patients, we state that NIRFLI is a high resolution exam to visualise the architectural changes of the superficial lymphatic network. We observed in every lymphedema NIRFLI abnormalities and in every healthy subject normal NIRFLI patterns. When clinical context and subjective symptoms are present, even without clinical oedema, we always observed NIRFLI abnormalities. Because the onset of secondary lymphedema occurs with a delay after surgery (mean time 27 months), we expect to observe those changes in part of our recruited patient in the near future.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-13-28

Title: Lipofilling of the axilla to reduce secondary lymphedema after axillary lymph node dissection

Vandermeeren L, Belgrado J-P, Vankerckhove S, Valsamis J-B, Feipel V, Rooze M, Moraine J-J, Hertens D, Carly B and Liebens F. CHU St-Pierre - Brussels Lymphology Clinic, Belgium; BEAMS, Belgium; Université Libre de Bruxelles, Belgium and CHU St-Pierre - ISALA Breast Clinic, Belgium.

Body: *Introduction*

Upper limb lymphedema remains a frequent complication (3-60%) of axillary lymph node dissection (ALND) for breast cancer. Part of these lymphedema present lymphatic but also venous impairment. During surgery, adipose tissue surrounding the axillary vein is partially or completely removed and the axillary sheath can also be damaged. This anatomical disruption could reduce the local hemodynamic condition, with as a consequence an increased microvascular filtration at the distal part of the affected limb. Patients with a venous impairment after ALND present a pitting edema at the level of the hand and forearm and other clinical signs that allows us to identify them. In order to reduce their edema, we propose an original and simple surgical approach that could partially restore the axillary hemodynamic impairment.

Material and Methods

BCRL patients with positive clinical signs for axillary hemodynamic changes underwent lipofilling under the axilla vein. Patients remain without any treatment nor sleeves during 10 days after surgery. Precise volumetry was performed the day before, the day after and 10 days after surgery (CE AK/13-06-75/4276AD, EudraCT n° 2015-001565-37). After 10 days, patients restart previous physical treatment and we continued to evaluate limb volume by volumetry. Subjective symptoms as numbness, heavy arm, pain and tension of the skin were evaluated.

Results

48 BCRL patients underwent lipofilling surgery. Edema volume reduced significantly in the majority of patients. This reduction was already observed directly after surgery, and was maintained before restart of physical treatment.

Subjective symptoms like heavy arm, numbness, and functional impairment of the upper limb in daily activities started to decrease directly after the operation. Most of the patients continued physical treatment, but felt that compression garments was not essential anymore to maintain edema at low level.

After 24 months of follow up, no complications were recorded.

Conclusion

In selected BCRL patients, lipofilling under the axillary vein improves local hemodynamic, reduces distal hyperfiltration and consecutively reduces part of the edema. Results of this pilot study need to be empowered by multicentric studies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-01

Title: International validation of the EORTC patient-reported outcome measure (PROM) in breast reconstruction (BRR): The EORTC QLQ-BRECON23 evaluating the psychometric properties and clinical effectiveness

Winters ZE E, Afzal M, Rutherford C, Holzner B, Rumpold G, da Costa Vieira RA A, Hartnup S, Filcroft K, Bjelic-Radusic V, Oberguggenburger A, Panouilleres M, Mani MR R, Catanuto G, Douek M, Kokan J and King MT T. University of Bristol, Faculty of Health Sciences, Bristol, Avon, United Kingdom; Quality of Life Office, School of Psychology, University of Sydney, Sydney, NSW, Australia; Medical University of Innsbruck, Innsbruck, Austria; Medical Psychology, Evaluation Software Development, University of Innsbruck, Innsbruck, Austria; Barretos Cancer Hospital, Sao Paulo, Brazil; St James's University Hospital, Leeds, United Kingdom; Poche Centre, University of Sydney, Sydney, NSW, Australia; Medical University Graz, Graz, Austria; Medical University of Innsbruck, Innsbruck, Austria; Besançon University Hospital, Besançon, France; Uppsala Academic Hospital, Uppsala, Sweden; Cannizzaro Hospital, Cannizzaro Hospital, Italy; Kings Health Partners Guy's Hospital, London, South East, United Kingdom; Cancer Resource Centre, Macclesfield Hospital, Manchester, United Kingdom and Director of Quality of Life Office, University of Sydney, Sydney, NSW, Australia.

Body: Introduction: PROMs are integral to assessing clinical effectiveness after BRR. The European Organization for Research and Treatment of Cancer (EORTC) BRR questionnaire (QLQ-BRR24) was developed for use alongside the EORTC PROMS: QLQ-C30 (cancer) and QLQ-BR23 (breast cancer).

Methods: Patients (n=438) from 28 centres across 9 countries covering 6 languages self-reported the: EORTC QLQ-C30, QLQ-BR23 and the QLQ-BRR24 before and after BRR including a debriefing questionnaire. Prospective patients (n=234) completed the QLQ-BRR24 at baseline (before mastectomy and BRR and at 4-8 months post BRR (response to change analysis, RCA). Cross-sectional patients (n=204) were at 1-5 years after BRR evaluating long-term quality of life (QOL) effects and test-retest reliability through repeated completion of QLQ-BRR24 (2-8 weeks apart). Scale convergent validity was tested using Pearson's correlations. Hypothesis-testing and clinical interpretability used known-group comparisons evaluating effect size and minimally important clinical differences.

Results: Patient groups: implant alone (n=176), donor flap (n=166) and control (mastectomy or staged-delayed, n=96). Six HRQL scales: i) sexuality; ii) treatment side effects; iii) donor symptoms; iv) breast cosmetic; v) nipple cosmetic; vi) satisfaction with surgery and 4 stand-alone questions (well-fitting bra, loss or preserve nipple, satisfaction with donor scars) were psychometrically tested. 16.1% (70/434) of women reported confusing, difficult or upsetting items. These comprised sexuality, the 1-week time window, and "coming to terms with disease or treatment". Five of the six QLQ-BRR24 multi-scales demonstrated good internal consistency (Cronbach's alpha: 0.76 - 0.93), except for treatment side-effects (arm tingling or fullness: Cronbach's alpha: 0.67). Test-retest reliability was good (intra-class correlations: 0.73 - 0.92). Low to moderate correlations ($r \leq 0.43$) between the scales of QLQ-BRR24 and QLQ-C30 and QLQ-BR23 indicated different/distinct constructs of the QLQ-BRR24 complementing existing questionnaires. Breast cosmetic scale showed a large effect (Cohen's $d=0.86$, $p<0.001$) between DIEP and LD flaps, and moderately significant ($p \leq 0.05$) effects for implant versus donor BRR, post-mastectomy radiotherapy and surgical complications. Most QLQ-BRR24 scales differentiated surgical complications. Sexuality and treatment side-effects showed small effect responsiveness. Well fitting bra was excluded based on weak EFA ($<.3$), floor/ceiling effects and counterintuitive differentiations within known group comparisons.

Conclusions: The internationally validated EORTC QLQ-BRECON23 evaluates effects of BRR in breast cancer patients, and complements the EORTC core breast cancer modules. Future studies should consider comparing it to the BREAST-Q, another questionnaire that assesses breast reconstruction outcomes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-02

Title: Post mastectomy breast reconstruction in elderly women: Complications and the impact of individual surgeons

Schoenbrunner AR R, Fero KE E, Boero IJ J, Matsuno R, Kronstadt N, Lance S, Reid C, Wallace AM M, Gosman AA A and Murphy JD D. University of California, San Diego School of Medicine; University of California, San Diego and University of California, San Diego.

Body: Purpose/Objective

In the United States over 40% of incident breast cancer diagnoses are in women over 65 years of age. Effective breast cancer treatments allow elderly patients to live long, healthy lives; questions regarding long-term quality of life are increasingly important. In women over 65, post-mastectomy breast reconstruction (PMBR) is uncommon with reported rates of 6-30%. The goal of this study is to report complication rates in elderly PMBR patients and to evaluate the impact of individual surgeons on PMBR in elderly patients.

Material/methods

We identified 19,417 Medicare beneficiaries diagnosed with localized breast cancer between 2005 and 2011 who underwent mastectomy. Medicare claims were used to identify PMBR, post-operative complications after PMBR (within 30 days of surgery), and long-term complications related to reconstruction (within three years following surgery). Mastectomy surgeon was identified from Medicare claims with surgeon characteristics identified through linkage to the American Medical Association (AMA) Masterfile.

Multi-level, multivariable logistic models clustered by surgeon and geographic area were used to determine the impact of surgeons on the likelihood of reconstruction. The intraclass correlation coefficient (ICC) and median odds ratio (MOR) were used to describe the relative impact of the individual surgeon. The ICC estimates the proportion of variability explained by the surgeon on PMBR rates. The MOR quantifies the likelihood of a patient having a different PMBR outcome if the patient were to change surgeons (or geographic area); it is directly comparable to odds ratios. Odds ratios (OR) were used to describe the impact of fixed demographic and clinical covariates.

Results

Among the entire cohort, 1,234 (6.4%) patients underwent PMBR. The post-operative complication rate was 8.4% and the long-term complication rate was 19.9%. Eighteen percent of the variability in PMBR use was attributed to the individual surgeon (ICC 0.181). The MOR for surgeon was found to be 1.85 (95% CI [1.70, 1.99]), indicating that a patient had an 85% chance of having a different outcome (receiving or not receiving PMBR) if the patient saw a different mastectomy surgeon. The MOR for geographic area indicated that a patient had a 32% chance of having a different outcome if the patient saw a surgeon in a different geographic area (1.32, 95% CI [1.17, 1.47]). Patients who were Asian, single, older, of lower socioeconomic status, and underwent radiation therapy were less likely to undergo PMBR. Patients who had pre-operative MRI or received chemotherapy were more likely to undergo PMBR. Patients who were treated by female surgeons or plastic surgeons were significantly more likely to undergo PMBR. Overall, the individual surgeon was the most predictive of PMBR, except for the use of pre-operative MRI and mastectomy surgeon's specialty being plastic surgery.

Conclusion

A small minority of older women undergo PMBR despite having low post-operative and long-term complication rates. The individual surgeon and geographic area significantly influences whether older breast cancer patients will undergo PMBR. Future research should focus on surgeon characteristics that may influence a patient's decision to undergo PMBR.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-03

Title: Does fat transfer increase the risk of breast cancer recurrence? A meta-analysis involving 2382 patients

Wazir U, Chehade HEH, Headon H, Oteifa M, Kasem A and Mokbel K. London Breast Institute, London, United Kingdom and Kuwait Cancer Control Centre, Al Sabah Medical District, Shuwaikh 70653, Kuwait.

Body: Introduction: Lipofilling is an increasingly popular technique for breast reconstruction following both mastectomy and breast conserving surgery. However, concerns remain over its oncological safety and its effect on cancer recurrence.

Methods: A systematic literature review and meta-analysis was carried out. Patients who had undergone mastectomy and patients who had undergone breast conserving surgery (BCS) were looked at separately in order to investigate whether the addition of lipofilling had a significant effect on locoregional recurrence rate.

Results: Eleven studies were used in the analysis yielding a total of 2382 patients. For patients undergoing mastectomy (mean follow up = 36.2 months: range=12-90) or BCS (mean follow up = 30.2 months: range = 12-60) , the addition of lipofilling was not found to significantly affect the locoregional recurrence rate.

Conclusion: This meta-analysis demonstrates that lipofilling is an oncologically safe procedure to be incorporated into breast reconstruction following either mastectomy or BCS for breast cancer. However a careful oncological follow up is recommended. In the future, more adequately- powered controlled clinical trials are needed in order to fully understand long term outcomes after lipofilling.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-04

Title: Exclusive fat grafting breast reconstruction after mastectomy: Feasibility and complications on 54 patients

Bordes V, Champion L, Lejeune F, Loirat Y, Boiffard F, Brillaud-Meflah V, Dravet F and Bouffaut A-L. ICO Rene Gauducheau, Nantes, France and Clinique BRETECHE, Nantes, France.

Body: Backgrounds: Autologous fat grafting has become a frequent, simply reproducible and low-risk technique in breast reconstruction. The potential risk of fat tissue transfer to the breast in oncologic patients remains to be discussed, but one must clearly distinguish the situation where there is a breast parenchyma left and where the whole gland has been removed, like in our study. Although lipotransfer has become very popular, only a limited number of case series have been reported up to date. The presented study describes an optimized treatment and complications for breast reconstruction after total mastectomy by lipotransfer alone.

Patients and methods: A retrospective study was performed in two French centers with five surgeons between February 2011 and June 2015, including 54 patients. Inclusion criteria were patients with prior breast cancer, treated by mastectomy and with a finished breast reconstruction with exclusive fat grafting. Exclusion criteria were prior reconstruction with implant or flap. We used two technics for liposuction: manual aspiration with syringes (pouret kit®) or waterjet assisted liposuction (body-jet®). The BRAVA® could be combined with the reconstruction. Our study had 2 main objectives: evaluate the factors influencing the number of surgical procedures and study the complications and carcinologic evolution. Statistical analysis was performed using stata 13.1 SE.

Results: We included 54 patients, 49 delayed reconstructions including two bilateral reconstructions and 5 immediate reconstructions. The morphologic data showed: a normal BMI for 70,3 % (38/54) patients and a bra cup A or B for 72,2 % (39/54). 39 patients had radiotherapy during the cancer treatment and the mean time between radiotherapy and reconstruction was 19 months. For the patients without radiotherapy, the mean time between mastectomy and reconstruction was 22 months. 6 patients (11%) were smoker or diabetic. We used manual aspiration in 37 patients (68,5 %) and hydro dissection in 17 patients (31,5%). 10 patients received BRAVA system in complement. We performed 231 fat grafting procedures with an average of 4,2 per patient. The mean total quantity of fat injected was 904 ml per patient with a mean quantity per procedure of 219 ml. The mean time between two procedures was 4,3 months. Only the prior radiotherapy treatment increases the number of fat grafting procedures significantly ($p=0,02$) and the use of hydro dissection with bodyjet decreases the number of fat grafting procedures significantly ($p=0,04$). We observed one failure of procedure requiring an implant and three patients with metastatic disease without local recurrence with a mean follow of 5, 2 years. We also observed 3% of infectious complications (7/231) and 19 patients presented fat necrosis (34%). Of these 19 cases of fat necrosis, 6 (11 %) required a surgery.

Conclusion: Autologous fat grafting can be proposed as an alternative for total reconstruction after mastectomy with a low level of complications and no local recurrence in our study. Only anterior radiotherapy increases the number of procedures probably due to fibrosis and lack of cutaneous flexibility.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-05

Title: Long term risk of explantation with Strattice™ assisted breast reconstruction, is it any different to submuscular reconstruction?

Wilson RL L, Kirwan CC C, Johnson RK K and Harvey JR R. University Hospital of South Manchester, Manchester, United Kingdom and University of Manchester, Manchester, United Kingdom.

Body: Introduction

The most recent meta-analysis published, containing only studies from 2011 onwards, reports acellular dermal matrix (ADM) assisted breast reconstructions are associated with a significant increase in risk of infection, seroma and mastectomy flap necrosis but not implant loss when compared to submuscular reconstructions. We hypothesised that implant loss associated with ADM-assisted reconstruction did not exclusively occur within the first 30 days after surgery and studies with short-term follow-up may underestimate the risk. We aimed to determine with long-term follow-up at what time point explantation occurs after Strattice™ ADM-assisted reconstruction and if it differs from traditional submuscular implant based reconstruction.

Methods

A retrospective case note review was completed for all immediate implant based reconstructions performed between 1st January 2009 and 31st December 2015 in a single tertiary centre in England. Implant losses, the timings and causes of loss were determined.

Results

In total there were 510 immediate implant based reconstructions performed in 373 patients, of which 135 were submuscular and 375 ADM-assisted.

In the ADM group a total of 22 (5.9%) implants were lost as a complication of their primary surgery. 14 implants were lost due to infection and eight due to wound breakdown. Implants were lost over a range of 14-661 days, median 76 days. Implant loss occurred within 30 days in six (27%), <90 days in 13 (59%) and over 90 days in nine breasts (41%). There were seven unplanned explantations, six were changed to autologous reconstructions (three for cosmetic reasons and three as a result of radiotherapy damage); one patient had a completion mastectomy for recurrent cancer.

In the submuscular group a total of 11 (8.1%) implants were lost as a complication of their primary surgery. Six implants were lost due to infection and five due to wound breakdown. Implants were lost over a range of 12-274 days, median 49 days. Implant loss occurred within 30 days in four (36%), <90 days in seven (64%) and over 90 days in nine breasts (36%). There were a further six unplanned explantations, two for pain and four for cosmetic reasons.

Comparing the two groups there were no differences in total implant loss rate or time to implant loss.

Conclusions

Implant loss within the first 3 months of ADM-assisted breast reconstruction is 3.5%; however, implant loss can occur more than 90 days after ADM-assisted breast reconstruction. Patients and clinicians should be aware that the risk of explantation continues for up to two years post-operatively with an ADM-assisted reconstruction whereas with submuscular coverage there were no implant losses beyond nine months follow-up. There were no differences in explantation rates between submuscular and ADM-assisted breast reconstructions.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-06

Title: A single-center report of 125 cases of incidental internal mammary lymph node biopsy in free abdominal flap breast reconstruction

Quan C, Huang N, Yang B, Zhang Y, Huang X, Chen J and Wu J. Fudan University Shanghai Cancer Center, Shanghai, China and Shanghai Shanghai Medical College, Shanghai, China.

Body: Objective

The aim of the current study is to determine the clinical value of incidental internal mammary lymph node biopsy in free abdominal flap breast reconstruction using internal mammary as recipient vessels and to investigate the risk factors of internal mammary lymph nodes metastasis.

Methods

The clinical data of all free abdominal flap breast reconstructions using internal mammary as recipient vessels performed from November 2006 to April 2016 in Department of Breast Surgery, Fudan University Shanghai Cancer Center were included into the study. The incidence of internal mammary lymph node biopsy and the rate of metastasis were described. The differences between groups were compared using χ^2 test. Univariate and multivariate Logistic regression analysis was conducted to evaluate risk factors of internal mammary lymph node metastasis.

Results

A total of 125 patients met the inclusion criteria, 89 (71.2%) of whom adopted unilateral immediate breast reconstructions. 64.8% of them were diagnosed with primary invasive breast cancer, while 27.2% were carcinoma in situ. According to AJCC TNM staging system, the percentages of patients with stage 0, I, II, III were 27.2%, 26.4%, 27.2% and 8.0%.

62 (49.6%) patients had internal mammary lymph nodes harvested. 6.4% (4/62) of those who adopted internal mammary lymph nodes biopsies were diagnosed with internal mammary lymph nodes metastasis, all in immediate breast reconstructions. 72 (57.6%) patients cut off costicartilage (the second or third costicartilage) during the exposure of internal mammary vessels. The excision of costicartilage is not associated with the harvest rate of internal mammary lymph node ($P>0.05$). All of the 4 patients upstaged their TMN stage of tumor after internal mammary lymph node metastasis and adopted adjuvant chemotherapy and radiotherapy. Up to May 2016, no recurrence or distant metastasis was reported.

In Logistic regression analysis, larger invasive tumor size and axillary lymph node metastasis were found to have correlations with internal mammary lymph node metastasis (larger invasive tumor size: $P=0.029$; axillary lymph node metastasis: $P=0.004$), while tumor location or immunohistochemical subtype had no correlations. In addition, axillary lymph node metastasis was proved to be a independent risk factor of internal mammary lymph node metastasis ($P=0.036$) while tumor size was not.

Conclusions

Internal mammary lymph nodes found incidentally during internal mammary recipient vessel exposure for free flap breast reconstruction could provide surgeons with important information for further treatment. This approach for internal mammary lymph node biopsy revealed an appreciable success rate and should be promoted in clinical practice. The size of invasive tumor and the axillary lymph node metastasis were associated with internal mammary lymph node metastasis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-07

Title: Neoadjuvant radiotherapy in mastectomy and immediate autologous free flap reconstruction. Findings from the primary radiotherapy and DIEP flap (PRADA) pilot study

Thiruchelvam P, Hadjiminias D, Cleator S, Wood S, Leff D, Jallali N, James S and MacNeill F. Imperial College School of Medicine, London, United Kingdom and The Royal Marsden Hospital, London, United Kingdom.

Body: Background:

The need for post mastectomy radiotherapy (PMRT), may preclude reconstructive surgeons from offering patients immediate, autologous reconstruction. This is due to historical evidence suggesting high rates of short- and long-term complications as well as poorer aesthetic outcomes. As the indications for PMRT broaden this practice denies an ever-increasing number of women the benefit of an immediate reconstruction.

Aim:

This pilot study evaluates the safety of offering radiotherapy *prior* to mastectomy and immediate DIEP flap reconstruction.

Methods:

Women planned for neoadjuvant chemotherapy (NACT), mastectomy (following unsuccessful breast conservation surgery (BCS) or upfront selection) and PMRT were offered a change in sequencing of RT at two academic breast surgery units in London, UK. Data was prospectively captured on 19 women, including: patient demographics, treatment details, tumour characteristics, oncological and post-operative outcomes. Operative parameters included unplanned return to theatre [RTT] <30 days, mastectomy skin flap necrosis, and evidence of wound infection at 5 days, 4 and 12 weeks post-operatively. All mastectomies, were performed by one of 3 breast surgeons (DH, FAM, DRL) using a circumareolar incisions with one patient undergoing a vertical pattern incision for skin reduction.

Results:

The cohort demonstrated a broad range of age, body mass index (BMI) and mastectomy weight [mean (range): age=46 years (28-72); BMI = 28.4 kg/m² (23-37.6) and specimen weight=678gm (257-1040)]. The mean time from completion of NACT to neoadjuvant radiotherapy (NART) was 31.1 days (9-49 days), and time from completion of NART to mastectomy and DIEP was 17.8 days (13-24 days). There was one unplanned RTT at 72 hours for an evacuation of haematoma, 1 revision of micro-vascular anastomosis, 1 clinical fat necrosis requiring formal excision and 1 wound debridement and primary closure for poor wound healing (vertical pattern skin reduction). There were no flap failures and no mastectomy envelope necrosis. With a mean follow-up of 16.2 months, there were no loco-regional recurrences, 5 distant relapses with mean presentation at 13.7 months and 2 breast-cancer related deaths at 13.9 and 22.2months respectively.

Conclusion:

This pilot study suggests that mastectomy and DIEP reconstruction is surgically feasible within 4 weeks of completing NART. In this small cohort of oncologically high-risk women with altered sequencing of RT we did not observe flap failure or post-mastectomy skin flap necrosis. A larger multicentre study with aesthetic assessment, PROMS and translational aspects is planned.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-08

Title: Immediate breast reconstruction is highly accepted by breast cancer patients undergoing mastectomy when routinely offered- Recent experience of an Australian tertiary oncoplastic breast unit

Cheung DSM SM, Trinh L, Edirimanne S and Eslick G. Nepean Hospital, Sydney, Australia and University of Sydney, Sydney, Australia.

Body: Introduction:

Despite recommendations by breast cancer guidelines, that immediate breast reconstruction (IBR) should be offered to all breast cancer patients considered for mastectomy national IBR rates have been reported as low as 12% in Australia. We report our oncological breast cancer unit experience on acceptance of IBR, where it is routinely offered for all the medical fit breast cancer patients.

Methods:

We retrospectively reviewed, prospectively collected data on IBR by 3 oncological breast cancer surgeons from 1st of September 2014 to 31st of March 2016. Patients were considered to be unfit for IBR if they were American Society of Anesthesiologist (ASA) Score 4 or 5, have unstable psychiatric illness, inflammatory breast cancer or high metastatic cancer burden. All the patients who were fit for IBR were offered both prosthetic and autologous IBR options.

Results:

137 patients underwent mastectomy operation, of whom 27 were considered unfit for IBR. Of the remaining 110 patients, 84 (76%) accepted IBR. Of the patients who had IBR 64 (76%) had single stage prosthetic, 15 (18%) two stage prosthetic and 5 (6%) autologous reconstructions. Those who accepted IBR were younger compared to those who didn't have IBR (median age: 47 yrs vs. 67 yrs, $p < 0.001$), and were more likely to be in current relationship (78.57% vs. 61.54%, $p = 0.06$). All the three surgeons had high IBR acceptance rates (70.45%, 71.43%, 84.44%) and patient insurance status did not influence IBR acceptance rate (public 57% vs. private 65%, $p = 0.47$). The majority of patients (25 of 26) who did not accept IBR stated that breast reconstruction was not important for their body image.

Conclusion:

Our recent data shows, in Australia, when routinely offered to breast cancer patients who are fit for reconstructive surgery by oncological breast cancer surgeons, IBR is highly accepted. Younger patients and those who are in current relationship, were more likely to accept IBR, whereas, individual perception of insignificance of breast reconstruction for the body image was the main determinant of not accepting IBR.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-09

Title: Quality of life assessed by BREAST-Q in patients with delayed immediate tissue expander based breast reconstruction with and without radiotherapy

Hamann M, Brunnbauer M, Scheithauer H, Hamann U, Pölcher M and Braun M. Red Cross Hospital Munich, Munich, Germany and LMU Munich, Munich, Germany.

Body: Introduction

Radiotherapy of the breast is known for having a negative impact on implant based breast reconstruction. Technical improvements, such as expander-implant based delayed immediate reconstruction (EIBR), seem to produce better results. The purpose of this study was to analyze the effects of prior radiotherapy (RT) and postmastectomy radiotherapy (PMRT) on patient-reported quality of life (QoL) in patients with EIBR compared to patients that underwent EIBR without any RT.

Material and methods

After a retrospective chart review, all patients with a history of EIBR at the Red Cross Hospital Munich were invited for a structured physical examination and interview. All operations were performed between 2008 and 2012 by two surgeons (H.U. and B.M.). To assess QoL, the BREAST-Q - post reconstruction module was used and results were compared between the following three cohorts: 1. patients without RT (n=65), 2. patients with PMRT (n= 27) and 3. patients with a history of breast irradiation before mastectomy (n=16). Patients with prior RT and a PMRT after local relapse were excluded.

Results

Of 161 patients eligible, 94 patients followed the invitation, 14 of them with bilateral EIBR. Baseline characteristics between participating and non-participating patients were comparable. The median follow-up time was 18.1 months. Most of the baseline characteristics (e.g. tobacco use, diabetes) were comparable between the cohorts. Application of (neo-) adjuvant chemotherapy was significantly more frequent in cohort 2. The category Satisfaction with the breast was numerically but not significantly better in cohort 1 than in cohort 2 (p 0,078) whereas the psychosocial well-being showed a trend in favor of cohort 2 (p 0,058). Satisfaction with breast was significantly better in cohort 1 than 3 (p 0,014) as well as Satisfaction with breast (implant only) (p=0,007). There was no difference for Satisfaction with outcome (p=0,666). Cohort 2 reached more points in all categories than cohort 3 although this difference was not significant. Of all patients, 92,7 %, 84,6 % and 78,6% (cohorts 1, 2 and 3) would undergo the operation again.

Conclusion

After short follow-up time, EIBR seems to be a feasible method for breast reconstruction resulting in a high QoL, even if PMRT is required. In patients with a prior RT, QoL is worse compared to the other groups. However, patients' acceptance of EIBR is still high. Prospective studies and long-term follow-up are required for definitive conclusions.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-10

Title: Shared decision-making approach in breast reconstruction in a developing country

Maciel-Miranda A, Gutierrez-Zacarias LM M, Cabrera-Galeana PA A and Bargallo-Rocha E. Instituto Nacional de Cancerología, Mexico City, Tlalpan, Mexico.

Body: Background

In Mexico breast cancer is the leading cause of cancer mortality in women.

The goal of reconstruction is restoration of patient's quality of life after cancer, including concerns about body image, sexuality, self-esteem and social life. Reconstruction is one of the most important determinants of long-term health.

Autologous reconstruction has been found to offer a better satisfaction in the long term, even these findings, a paradigm shift toward implant reconstruction has been described previously.

Shared decision making (SDM) is a collaborative process that allows patients and their providers to make health care decisions together. It takes into account the best clinical evidence available, as well as the patient's values and preferences. In medical treatments where several options have been found, SDM has been a useful tool, increasing patient's satisfaction and better outcomes. Through this process of informing and involving the patient, high-quality decisions that align with patient preferences are achievable.

Methods

Evaluation of particular case was made, with personalized approach depending on breast cancer stage and mastectomy type planned. On first consultation basic information about different techniques was offered: pros and cons on surgical time, recovery time, long term complications, sensation and physical appearance. We offered a second consultation if the patient wanted to go deeper in the information we presented, or even if she wanted to look out for more information on the internet and other sources. When an abdominal free flap was planned, we saw the patient in a second consultation to review the CT-angio, and to establish the definitive flap option.

For risk estimation we used www.brascore.org website, and talk about it with the patient. Then patient and plastic surgeon made decision on breast reconstruction technique. BREAST-Q questionnaires were applied in pre and postoperative setting.

Results

From 06/2014 to 06/2016 we performed 150 breast reconstructions with this SDM approach with the following techniques: 64% expander, 15% direct to implant, 11% DIEP flap, 6% latissimus dorsi flap, 3% Becker implant, 1% TRAM flap.

BREAST-Q questionnaires showed 100% think breast reconstruction is better than do not reconstruct, and 100% would recommend reconstructive surgery. 91.7% felt included in the decision process. Satisfaction with breast (preoperative vs postoperative) 66.21 vs 81.13, Satisfaction with outcome 85.06, Psychosocial well being 80.75 vs 88.35, Physical well-being 74.92 vs 68.73, Sexual well-being 66.66 vs 72.84, Satisfaction with information 86.86, Satisfaction with surgeon 98.73, Satisfaction with medical staff 96.2.

Analysis

Implant based reconstruction (82%) was preferred due to: simplicity, less overall risk, and giving more importance to cancer treatment and leaving reconstruction in second place. It could also be a short-term vision regarding benefits, in spite of shared information.

Aesthetic improvement of the abdominal area seems to be a factor to decide for autologous abdominal flap among young adult women (7%).

Possibility of pregnancy should be considered among young women. In our breast reconstruction program for young women with breast cancer, egg preservation is offered, so abdominal flaps should be delayed after a possible pregnancy.

Conclusions

We need to encourage SDM in breast reconstruction. We need information systems available to patients prior to breast reconstruction like educational meetings, giving healthcare professionals feedback, giving healthcare professionals learning materials, and using patient decision aids.

Shared decisions leads to better outcomes and high patient's satisfaction in breast reconstruction.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-11

Title: Risk factors affecting post-operative complication after immediate reconstruction with implant for operable breast cancer patients

Park SJ, Choi JH, Lee MH, Jung S-Y and Lee ES. Center for Breast Cancer, Research Institute and Hospital, National Cancer Center, Goyang, Gyeonggi, Republic of Korea.

Body: Purpose :

Immediate breast reconstruction (IBR) with implant after nipple-areola complex (NAC) sparing mastectomy is an increasing treatment for breast cancer patients. Old age, obesity, smoking history, large volume of implant and adjuvant oncologic therapies have been known as risk factors for post-operative complication after IBR. However, less is known about the risk factors after IBR with implant in Asian women with relatively low body mass index (BMI), small volume of breast and less history of smoking. We analyzed the risk factors for post-operative complications after IBR with implant in patients receiving NAC sparing mastectomy.

Methods :

We investigated a prospectively maintained database from 2012 to 2015 at National Cancer Center. A total of 278 breast reconstructions (230 unilateral, 24 bilateral) in 254 patients were performed by a single surgeon; 99.3% (276 of 278 breasts) were oncologic, and 0.7% (2 of 278 breasts) were prophylactic mastectomy. For each patient, we collected data on age, BMI, history of smoking and diabetes mellitus, volume of implant, adjuvant oncologic therapies, distances from tumor to nipple and skin, imprecise magnetic resonance image (MRI) finding (no discernible enhancing, non-mass enhancement, and multifocal tumor), and use of acellular dermal matrix (ADM).

Results :

Mean age was 44.8 ± 7.6 years and mean BMI was 22.5 ± 2.9 kg/m². Patients with smoking history was 4.3% (n=11). Mean implant size was 242.3 ± 57.7 mL. Patients underwent chemotherapy in 39% (n=99) and radiotherapy in 29.1% (n=74). Compared to western studies, our patients showed lower BMI, less history of smoking, and smaller size of implant insertion. Overall post-operative complication rate was 8.3% (n=23) and the complication rate was similar to the results of western studies; which included wound dehiscence (4.3%, n=12), infection (2.9%, n=8), and implant loss (2.9%, n=8). The use of ADM was independently associated with post-operative complications (30.0% in patients used ADM vs 6.6% in patients without ADM, respectively; OR, 6.016, 95% CI, 2.018-17.939; p=0.001). Other factors were not significantly related to post-operative complications.

Conclusions :

Our study is the largest study to investigate the risk factors for post-operative complications after IBR with implant in Asian countries. Compared to western studies, the patients had lower BMI, less history of smoking and smaller size of implant. Although known risk factors, such as smoking, obesity and size of implant, were not significantly associated with post-operative complication in our study, the incidence of post-operative complication was similar to that of western studies. These results might be caused by higher post-operative complication rate after use of ADM compared with western studies. Further study is warranted to evaluate the impact of ADM on post-operative complication on Asian countries.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-12

Title: Oncologic outcomes of immediate breast reconstruction after neoadjuvant chemotherapy in breast cancer patients: A matched case control study

Ryu JM, Lee JE, Kim SW, Yu J, Rayzah M, Lee SK, Mansoor A, Bae SY, Park S, Paik H-J, Kim I, Bang SI, Jeon BJ, Mun G-H and Pyon J-K. Samsung Medical Center, Seoul, Korea.

Body: Introduction: Although the indication for total mastectomy (TM) with immediate breast reconstruction (IBR) has been expanded, IBR after neoadjuvant chemotherapy (NACT) is still controversy. We assumed that TM with IBR after NACT is feasible surgical treatment in breast cancer patients. **Methods:** A retrospective review of breast cancer patients who underwent TM with IBR after NACT between 2008 and 2015 at a single center was conducted. These cases were matched by 1:5 to patients who underwent mastectomy alone after NACT. Matching variables included age, clinical T and N staging before NACT, response to NACT, and pathologic staging after NACT. Pathological stage was followed by seventh American Joint Committee on Cancer (AJCC) classification. **Results:** Overall, 31 patients were identified in the TM with IBR group (Study group) and 85 patients (Control group) were matched. In the study group, 13 (41.9%) patients underwent nipple-sparing mastectomy (NSM) and 18 (58.1%) underwent skin-sparing mastectomy (SSM). Median follow-up duration was 29.2 (7-31) and 38.8 (11-85) months for the study and control group, respectively. Median age was 37.0 (26-57) and 40.0 (24-56) years for the study and control group, respectively. The clinicopathologic characteristics of both groups are summarized in Table 1.

Clinicopathologic characteristics

Variables	Control group (n=85)	Study group (n=31)	p-value
Age, years (matching variables)			0.890
≤35	15 (17.7)	9 (29.0)	
36-50	61 (71.8)	21 (67.7)	
51≥	9 (10.6)	1 (3.2)	
BMI, m2/kg			0.130
25≤	62 (72.9)	28 (90.3)	
26-30	18 (21.2)	2 (6.5)	
30>	5 (5.9)	1 (3.2)	
Histology			0.326
Ductal carcinoma in situ	2 (2.4)	3 (9.7)	
Invasive ductal carcinoma	74 (87.1)	28 (90.3)	
Invasive lobular carcinoma	2 (2.4)	0 (0)	
Others	7 (8.2)	0 (0)	
Multiplicity			0.063
yes	19 (22.6)	12 (40.0)	
no	65 (77.4)	18 (60.0)	
Lymphovascular invasion			0.161
yes	33 (39.3)	17 (54.8)	
no	51 (60.7)	14 (45.2)	
Nuclear grade			0.317
Low	10 (11.9)	1 (3.3)	
Intermediate	27 (32.1)	14 (46.7)	
High	47 (56.0)	15 (24.2)	

Pathologic T stage (matching variable)			0.154
T1	7 (8.2)	6 (19.4)	
T2	29 (34.1)	15 (48.4)	
T3	31 (36.5)	4 (12.9)	
T4	18 (21.2)	6 (19.4)	
Pathologic N stage (matching variable)			0.494
N0	36 (42.4)	13 (41.9)	
N1	23 (27.1)	13 (41.9)	
N2	16 (18.8)	4 (12.9)	
N3	10 (11.8)	1 (3.2)	
Estrogen receptor			0.608
positive	49 (57.7)	15 (48.4)	
negative	36 (42.4)	16 (51.6)	
Progesterone receptor			0.291
positive	40 (47.1)	10 (32.3)	
negative	45 (52.9)	21 (67.7)	
HER2 status			0.345
amplification	29 (34.1)	10 (32.3)	
not amplification	56 (65.9)	21 (67.7)	
Clinical T-stage (matching variable)			0.897
cT1	2 (2.4)	1 (3.2)	
cT2	31 (36.5)	12 (38.7)	
cT3	46 (54.1)	16 (51.6)	
cT4	6 (7.1)	2 (6.5)	
Clinical N stage (matching variable)			0.947
cN0	3 (3.5)	1 (3.2)	
cN1	20 (23.5)	10 (32.3)	
cN2	36 (42.4)	10 (32.3)	
cN3	26 (30.6)	10 (32.3)	
Response (matching variable)			1.000
Partial response	64 (75.3)	27 (29.7)	
Stable disease	21 (24.7)	4 (12.9)	

Disease-free survival ($p=0.520$), local recurrence-free survival ($p=0.610$), distant metastasis-free survival ($p=0.795$), and over survival ($p=0.971$) did not differ significantly between two groups. **Conclusion:** TM with IBR after NACT is feasible surgical treatment option in breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-13

Title: Total mastectomy and immediate breast reconstruction for breast cancer: A ten-year Canadian single institution experience

Younan RJ J, Al Khaldi M, Maalouf C, Guez M, Boulva K, robidoux A, Nassif E, Poljicak M, BouMerhi J, Bernier C and Patocskai E. University of Montreal Hospital Center, Montreal, QC, Canada.

Body: Fear of high local recurrence rate associated with immediate breast reconstruction (IBR) following total mastectomy for breast cancer might be a contributing factor to the low rate of immediate reconstruction performed in Quebec. The aim of this study was to demonstrate the oncological safety of total mastectomy with immediate breast reconstruction.

A retrospective chart review of all patients who underwent total mastectomy with immediate breast reconstruction at the University of Montreal Hospital Center between 2006 and 2015 was performed.

375 patients (420 cases) met the inclusion criteria. The mean age was 51.5 years (25-77). The median follow-up was 45.6 months (0.4-115.2). Clinical cancer staging was done according to the American Joint Committee on Cancer (AJCC) criteria. 349 cases (83.1%) were classified as Stage < cIIb and 71 cases (16.9%) as ≥ cIIb. 73 patients (19.5%) received neoadjuvant chemotherapy, 113 (30.1%) received adjuvant chemotherapy and 91 (21.7%) received postoperative radiotherapy. Only 4 patients (3.5%) had a significant delay in receiving adjuvant chemotherapy and 4 patients (4.4%) had a delay in initiation of radiotherapy. In total, there were 12 (2.6%) local recurrences and 29 (7.7%) distant recurrences.

The results of this study demonstrate a low rate of local recurrence that is comparable to the current literature. Total mastectomy in association with immediate breast reconstruction is therefore an oncologically safe approach for the treatment of breast cancer and should be more widely adopted by medical centers throughout Quebec.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-14-14

Title: Endoscopic 3D latissimus dorsi muscle harvesting for immediate breast reconstruction. Results of our 7 patients experience in Antoine Lacassagne Nice Cancer Center

Barranger E and Dejode M. Cancer Center Antoine Lacassagne, Nice, Provence Alpes Côte d'Azur, France.

Body: Introduction :

The harvest of the latissimus dorsi muscle for the realization of immediate breast reconstruction induces a large dorsal scar measuring up to 20 centimeters, while only the muscle is used. We present here the results of our experience of endoscopic harvesting of the latissimus dorsi muscle using 3D technology. Our main objective is to reduce the dorsal scar and second assess the functional consequences for patients.

Methods:

Between December 1st, 2015 , and June 1st, 2016, there were 7 patients who underwent latissimus dorsi endoscopic harvesting, with 3D technology, for an immediate breast reconstruction after a skin-sparing or nipple sparing mastectomy.

Results:

4 patients had latissimus dorsi muscle with breast implant and 3 patients by latissimus dorsi muscle only. The age average was 55 years (range 45-70). The mean length of surgery was 250 minutes (range 150- 380). The mean length of hospitalization was 7 days (range 4-12 days). One patient treated for local recurrence had skin necrosis because of previous radiotherapy. Donor site seroma was the main post surgery drawback. Patients on average benefited from 3 evacuations (range 1-5) of 850ml (range 640-1270ml). Acceptance of the reconstructed breast was good in six cases, and poor in one case. Only one patient reported discomfort at the donor site 1 month after surgery.

Conclusion:

The endoscopic 3D latissimus dorsi flap harvesting allows to reduce donor site scar. There is no significant difference concerning complications or post-operative pain and functional discomfort between the endoscopic and classic harvesting procedure.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-15-01

Title: Prognostic significance of CD8+ tumor-infiltrating lymphocytes (TILs), CD4+ TILs and TILs in male breast cancer

Abdeljaoued S, Bettaieb I, Adouni O, Goucha A, Ghozzi R, Bouzaiene H, Hdiden M, Ben Hassouna J, Makhoulouf R, El Benna F, Boussem H, Rahal K and Gamoudi A. Salah Azaïz Cancer Institute, Tunis, Tunisia and Ariana Hospital, Ariana, Tunisia.

Body: Background

Several studies have demonstrated that the presence and the amount of tumor-infiltrating lymphocytes (TILs) are a potential biomarker of prognostic significance in some solid tumors. Data suggests that high levels of TILs are associated with favorable prognosis. However, whether TILs have any indication for prognosis in male breast cancer (MBC) patients remains unknown. In this study, we investigated the relationship between the expression and degree of CD8+ TILs, CD4+ TILs, and TILs and evaluated the prognostic value of these three factors in MBC.

Methods

We retrospectively identified 110 male breast cancer patients diagnosed between 2000 and 2013 at Salah Azaïz Cancer Institute. Stromal (str) CD8+ and CD4+ TILs were evaluated immunohistochemically. Two pathologists independently evaluated TILs levels using H&E stained slides following 2014 International TILs Working Group guidelines. Kaplan Meier and univariate and multivariate Cox regression analysis were applied to determine the association of CD8+ TILs, CD4+ TILs and TILs with clinicopathological features and overall survival (OS).

Results

The median for presence of str-CD8+TILs was 7% (0%-40%), str-CD4+TILs was 2% (0%-20%) and str-TILs was 10% (1%-40%). Only 12% of MBC patients had high str-CD8+TILs and 11% had high str-CD4+TILs. Fifty three percent had low str-TILs and 47% had moderate str-TILs. No lymphocyte predominant breast cancer was identified. Regarding intrinsic molecular subtypes, TNBC subtype and HER2 enriched tumors compared with Hormone receptor positive tumors had higher median levels of str-CD8+TILs, str-CD4+ TILs and str-TILs at diagnosis. However, increased level of str-CD8+ TILs, str-CD4+TILs and str-TILs weren't associated with improved OS in IHC subtypes ($p=0.597$, $p=0.498$ and $p=0.456$ respectively). On univariate analysis, higher levels of str-CD8+TILs, str-CD4+ TILs and str-TILs were associated with better OS ($p=0.035$, $p=0.043$ and $p=0.040$ respectively). Multivariate analysis identified str-CD8+ TILs and str-TILs as independent prognostic factors for OS ([HR= 0.851 (0.706-0.997), $p=0.000$] and [HR= 0.69 (0.43-0.96), $p=0.045$] respectively) but not str-CD4+ TILs ($p=0.276$). A high level of str-TILs was correlated with high str-CD8+ ($p = 0.025$).

Conclusion

The level of TILs in male breast cancer is low, suggesting prominent immune regulation/suppression in this disease. Str-CD8+ T cells and str-TILs represent potential prognostic markers in male breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-16-01

Title: Immune characterization of inflammatory breast cancer and correlation to pathological complete response

Reddy SM M, Wargo JA A, Reuben A, Reuben J, Woodward W, Ueno N, Mittendorf EA A and Krishnamurthy S. University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background:

Treatment of inflammatory breast cancer (IBC) includes neoadjuvant chemotherapy (NAC) followed by mastectomy and radiation. Responses are limited however with low pathological complete response (pCR) rates and poor survival. Recent RNA expression studies suggest that activated T cell signaling pathways and immunomodulatory markers such as PD-L1 are associated with a higher pCR rate in IBC; however comprehensive studies of tumor infiltrating lymphocytes (TIL) and protein expression of immunomodulatory molecules are lacking. There is a critical need therefore to study molecular and immune determinants of therapeutic response in IBC, with the goal of identifying biomarkers and actionable strategies to improve treatment outcomes.

Methods:

Baseline core biopsies from 36 IBC patients, 22 with stage III and 14 with stage IV disease were evaluated. Of these, 21 stage III and 10 stage IV patients underwent mastectomy following NAC, the latter for palliative purposes. Tumor subtype distribution was 14 patients with HER2-/HR-, 6 with HER2+/HR-, 11 with HER2-/HR+, and 5 with HER2+/HR+ disease. TIL infiltration in the tumor stroma was quantified on H&E slides based on consensus guidelines as well as by immunohistochemistry (IHC) staining for CD8. PD-L1 expression in the TIL and invasive tumor was evaluated by IHC in tumors with >1%TIL.

Results:

Stromal TIL were found in the invasive tumor on pretreatment biopsies in 26 (72%) patients, with TIL percentages ranging from 1% to 60% (mean=11.6; sd=13.8); of note, 1 patient biopsy sample only had tumor emboli on the tissue block and therefore was not evaluable. Higher TIL infiltrate was noted in stage III versus stage IV disease (mean TIL 11.6% versus 3.5%, p=0.028). Mean TIL infiltrate was 11.5% in HER2-/HR-, 10.0% in HER2+/HR-, 10.4% in HER2-/HR+, and 3.6% in HER2+/HR+ tumors (p=NS). At mastectomy, 7/21 stage III patients and 1/10 stage IV patients achieved a pCR. Mean TIL was 13.4% in the pCR group versus 8.2% in the non-pCR group (p=0.37) CD8 and PD-L1 staining was performed on samples with >1%TIL (n=15, of which 14 samples were available for additional staining). An average of 42% of TIL stained positive for CD8 (range 10-80%). There was no significant relationship between %CD8 and pCR, stage, or receptor status. None of these 14 patients demonstrated membranous PD-L1 positivity but all had focal weak cytoplasmic staining in the lymphocytes.

Conclusions:

Differences exist in the presence of stromal TIL in distinct groups within IBC (stage III versus stage IV disease and across histologic subtypes) and may contribute to differential responses to therapy. When comparing these results to published non-IBC literature (FinHER trial), our IBC patient cohort had lower TIL infiltrate in several histologic subtypes (HER2-/HR- 11.5% vs 25%, p=0.015), HER2+/HR-(10% vs 20%, p=0.10), and HER-/HR+ disease (3.6 vs 7.5%, p=0.01); TIL was comparable for HER2+/HR+ disease. Additional studies are underway (including multiplex analysis of myeloid and lymphoid markers, T cell receptor sequencing, and molecular profiling) in pre-treatment and surgical samples to better understand mechanisms of treatment response and resistance.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-16-02

Title: Nanotheranostics using plasmonic gold nanostars to target inflammatory breast cancer cells and tumor emboli

Shammas RL L, Fales AM M, Crawford BM M, Hollenbeck ST T, Vo-Dinh T and Devi GR R. Duke University School of Medicine, Durham, NC; Duke University, Durham, NC; Duke, Durham, NC and Duke, Durham, NC.

Body: Introduction: Due to the aggressive nature of inflammatory breast cancer (IBC) and insensitive imaging techniques, patients diagnosed with IBC have a poor prognosis. IBC's unique ability to form emboli leads to rapid growth and distant invasion. New therapies aimed at disrupting the formation of tumor emboli are needed. Gold nanostars (GNS) are unique nanoparticles that can be imaged in real time with high sensitivity, and are rapidly endocytosed into cancer cells. Due to their sharp spikes, GNS exhibit plasmonic properties, which strongly enhance the electromagnetic field upon laser excitation. In addition to their use as highly intense and stable fluorescent labels, these nanoparticles can be photothermally activated to trigger cellular ablation. The use of GNS as an imaging and therapeutic modality for IBC should be investigated.

Methods: IBC (SUM-149/SUM190), non-IBC (BT474M1/MD-MBA-231), and drug resistant isotype variant (rSUM-149) cell lines were incubated with GNS, stained with Hoechst33342, and imaged with multiphoton microscopy (MPM). The dose and time dependent effects of GNS on SUM-149 proliferation was assessed with MTT assays. Photothermal treatment was performed on GNS-labeled IBC, non-IBC, and drug resistant cancer cell lines. The effects of photothermal therapy on cell viability were assessed using fluorescein diacetate and propidium iodide. Using a tumor emboli model, SUM-149, rSUM-149, and SUM-190 tumor emboli were labeled with GNS on the 3rd day of embolic maturation. Emboli were sectioned, stained with H/E, and imaged with MPM to demonstrate the depth of GNS penetrance. The potential for photothermal ablation of the GNS-labeled tumor emboli was assessed using various laser intensities. Propidium iodide was used to examine emboli viability following treatment.

Results: In all cell lines, GNS displayed rapid cellular uptake without nuclear involvement. MTT assay showed that GNS concentrations of 0.15 and 0.20 nM caused decreases in cell proliferation at 6 and 12 hours. Proliferative capacity was unaltered at 24 hours for all concentrations, however this may be due to spectrophotometric interference with high intracellular GNS concentrations. Live/dead staining confirmed effective photothermal treatment in all cultures with a clear zone of cellular death. For tumor emboli studies, GNS allowed bright fluorescent monitoring of tumor emboli using MPM, and cross sectional imaging demonstrated GNS penetrance into the embolic core. Photothermal ablation of GNS-labeled tumor emboli was successfully demonstrated following laser irradiation. Cell death was confirmed with propidium iodide.

Conclusion: Gold nanostars provide a highly fluorescent intracellular label for cancer cell lines, and tumor emboli without significantly altering cell proliferation. Furthermore, the inherent optical properties of the GNS allows for a combined therapeutic application following photoactivation. This is the first study to demonstrate the nanotheranostic application of GNS in IBC tumor emboli. Prior studies have shown the effectiveness of photothermal ablation in *in vivo* sarcoma models; we are currently extending our studies to an IBC mouse model.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P3-17-01

Title: Long-term rates of breast preservation after breast-conserving therapy for ductal carcinoma in situ

Rakovitch E, Nofech-Mozes S, Hanna W, Gu S, Fong C, Tuck A, Sengupta S, Elavathil L, Jani P, Done S, Miller N, Youngson B, Bonin M, Chang M and Paszat L. Sunnybrook Health Sciences Centre, University of Toronto, Toronto, ON, Canada; Institute for Clinical Evaluative Sciences, Toronto, ON, Canada; Kingston General Hospital, Kingston, ON, Canada; University Health Network, Toronto, ON, Canada; Thunder Bay Regional Health Sciences Centre, Thunder Bay, ON, Canada; Henderson General Hospital, Hamilton, ON, Canada; Sudbury Regional Hospital, Sudbury, ON, Canada; London Health Sciences Centre, London, ON, Canada and Mount Sinai Hospital, Toronto, ON, Canada.

Body: Background: Despite evidence that radiotherapy (RT) after breast-conserving surgery (BCS) for ductal carcinoma in situ (DCIS) halves the risk of recurrence, the benefit of RT in the management of DCIS continues to be a matter of controversy. One argument against the use of RT after BCS is that patients who develop ipsilateral local recurrence (LR) can be salvaged with further breast-conserving surgery such that the omission of RT will lead to high rates of breast preservation while minimizing exposure to RT. Breast preservation is an important determinant of quality of life for women with early stage breast cancer and DCIS. Yet the management of LR and the impact of RT on the resultant long-term risks of bilateral breast preservation in a population of women with DCIS are unclear. We assessed the treatment of LR, the impact of RT on the use of salvage mastectomy and the long-term risks of bilateral breast preservation achieved in a population of women with DCIS treated with BCS alone or BCS+RT.

Methods: A population-based analysis of women diagnosed with DCIS from 1994-2003 treated with BCS alone or BCS+RT with pathology review. Treatment and outcomes, including the development of LR and contralateral breast events, were determined by administrative databases with validation by review of operative or pathology reports. Median follow-up was 10.2 years for cases treated by BCS alone, 11.6 years for those treated by BCS+RT. We used a propensity-adjusted Cox proportional hazards model to evaluate factors associated with the use of salvage mastectomy for LR and to evaluate factors associated with any mastectomy. We assessed the risk of long-term breast preservation by calculating the KM 10-year risk of ipsilateral mastectomy and any mastectomy.

Results: The population cohort includes 3303 women with pure DCIS; 1649 (50%) were treated by BCS alone, 1654 (50%) received BCS+RT. Women treated with RT had more high risk features of DCIS than those treated by BCS alone. LR developed in 343 (21%) women treated by BCS alone and in 257 (15.5%) women treated by BCS+RT ($p < 0.01$). Most women who developed LR received salvage mastectomy, irrespective of age at diagnosis and histology. Salvage mastectomy was used in 57.4% (197/343) of cases that recurred after BCS alone and in 67.6% (173/257) that recurred after BCS+RT. The likelihood of receiving salvage mastectomy for LR was similar in patients initially treated by BCS+RT vs. those initially treated BCS alone. Most (90%) of mastectomies were performed for a LR. Overall, individuals initially treated by BCS+RT had a 29% lower probability of having a mastectomy at 10 years compared to those treated by BCS alone (HR=0.71, 95%CI: 0.60,0.84, $p < 0.0001$). The 10 year mastectomy-free survival rates are 82.7% for women initially treated by BCS alone and 87.3% for those treated by BCS+RT ($p = 0.0096$).

Conclusion: Women who received RT after BCS for DCIS experience a greater likelihood of long-term bilateral breast preservation. This is attributable to the lower risks of LR and that most local recurrences after breast-conserving therapy are treated by salvage mastectomy. Long-term breast preservation should be considered in discussions weighing the benefits and risks of RT after conservative surgery for DCIS.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-17-02

Title: Gene expression differences between ductal carcinoma in situ with and without progression to invasive breast cancer

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Body: Background

The mechanism behind progression of ductal carcinoma in situ (DCIS) to invasive breast cancer (IBC) remains unknown. The aim of our study was to increase our understanding regarding molecular alterations driving DCIS progression by comparing gene-expression patterns between patients with pure DCIS and patients with synchronous DCIS and IBC.

Methods

In this retrospective study, we included patients with extensive pure DCIS (n=12), defined as > 5 cm, as a representation of biologically indolent lesions with limited invasive capacity. These cases were matched with patients with a limited DCIS component, defined as < 1 cm, and synchronous IBC (n=12), representing lesions with a high invasive potential. Matching was based on age and surrogate DCIS subtypes. Gene expression profiling, using 93 tumor-specific target genes, was performed to identify transcriptional differences between the DCIS components of these two groups. The identified genes were validated by immunohistochemistry.

Results

In total, for 9 genes there was a significant difference in gene expression between patients with pure DCIS and patients with DCIS and synchronous IBC. The majority of these 9 genes were significantly higher expressed in DCIS samples with IBC, including *PLAU* ($P=0.002$), *COL1A1* ($P=0.006$), *KRT81* ($P=0.009$), *S100A7* ($P=0.015$), *SCGB1D2* ($P=0.023$), *KRT18* ($P=0.029$) and *NOTCH3* ($P=0.044$), while *EGFR* and *CXCL14* showed a significantly higher expression in pure DCIS ($P=0.015$ and $P=0.028$ respectively). Based on these 9 genes, unsupervised hierarchical clustering-analysis revealed distinct clustering of patients with pure DCIS and patients with DCIS and synchronous IBC. Immunohistochemical analyses are in progress.

Conclusion

This pilot study suggests that patients with pure DCIS have a significant different gene expression pattern as compared to patients with DCIS and synchronous IBC. If these results can be validated in an independent cohort, these differently expressed genes could be used to predict progression in individual patients diagnosed with DCIS. Furthermore, these genes may pinpoint driver pathway(s) that play an important role in the progression of DCIS to IBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-17-03

Title: DCIS and the risk of breast cancer death - A case control study

Wadsten C, Garmo H, Fredriksson I, Sund M and Wärnberg F. Sundsvall Hospital, Sundsvall, Sweden; Umeå University, Umeå, Sweden; Uppsala University, Uppsala, Sweden; Regional Cancer Center, Uppsala-Örebro, Uppsala, Sweden; Cancer Epidemiology & Population Health, Kings College, London, United Kingdom and Karolinska Institutet, Solna, Sweden.

Body: *Introduction*

The risk of breast cancer death after a primary ductal carcinoma in situ (DCIS) is less than 2 % after 10 years. Whereas in situ recurrences do not influence survival, a 17-fold elevated risk of breast cancer specific mortality has been shown for invasive recurrences. Adjuvant radiotherapy (RT) effectively reduces recurrences after breast conserving surgery (BCS) for DCIS, but no studies have been able to demonstrate a survival benefit from adjuvant RT treatment or from choosing mastectomy instead of BCS. Here patient and tumour related risk factors for breast cancer death in women with a pure primary DCIS were studied.

Patients and methods

Women registered with a primary DCIS, between 1992-2012 in three of Sweden's health care regions with a population of approximately 5.2 million, were enrolled in a nested case-control study. Out of 6,964 women with DCIS, 96 patients who later died from breast cancer were identified. Four controls per case (n=318) were randomly selected by incidence density sampling. We retrieved medical records and pathology reports and calculated OR with 95% CIs for various variables using conditional logistic regression.

Results

Of the 96 cases, 10 patients developed distant metastasis without a known local recurrence. In 56 patients death was preceded by an invasive ipsilateral recurrence and in 3 patients by a recurrent ipsilateral DCIS. Seven patients had invasive breast events in both the ipsilateral and the contralateral breast. Seventeen patients had contralateral invasive breast cancer and 3 patients contralateral DCIS.

Multifocality and tumour size over 25mm (OR 2.6 (1.6 to 4.2)), positive or uncertain margin status (OR 2.8 (1.6 to 4.9)) and detection outside screening (OR 2.1 (1.2 to 3.9)) increased the risk of breast cancer death in univariate analysis, when adjusted for age and year of diagnosis. Suspicion of micro-invasion and nuclear grade 3 was associated with a nonsignificant increased risk, OR 1.8 (0.6 to 5.0) and 2.6 (0.9-6.5), respectively. The risk was not affected by age or treatment. Tumour size and margin status remained significant in the multivariable analysis, when adjusted for treatment and for contralateral breast cancer (OR 2.0 (1.2 to 3.7)).

Conclusion

In the present study, large tumours and positive or uncertain margin status were significant risk factors for later breast cancer death after a primary DCIS. More extensive treatment was not related to a lower risk. The significance of tumour biology and nuclear grade will be further examined and evaluated.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-17-04

Title: Presentation of a Bayesian decision model for the treatment of ductal carcinoma in situ (DCIS) of the breast

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Body: INTRODUCTION: Breast carcinoma (BC) is the most common cancer in women worldwide with an increasing incidence by 2% annually, representing a major health problem in developed countries. The National Health Systems are focused on early diagnosis to minimize the fatal consequences of the disease, conducting screening mammography in women aged 40 to 70 years. However, early diagnosis has dramatically increased the diagnosis of ductal carcinoma in situ (DCIS).

Medical treatment decisions are based on Clinical Practice Guidelines (CPG), which provide physicians general recommendations to help obtain the best optimal patient care. Many studies have reported improper use in routine clinical practice as they do not take into account the uncertainty of the decision or the risks and benefits involved.

Thus, the increased frequency of patients diagnosed with DCIS secondary to breast screening programs and the deficits in the current making decision process based on GPC, are the main reasons we have taken into account to build a System Decision Support (SDS) for the treatment of patients with DCIS.

MATERIAL AND METHODS: The research centers involved in this study were the Valencian Institute of Oncology (IVO) and the Polytechnic University of Valencia and within the Institute for the Application of Information Technology and Advanced Communications (ITACA). Researchers from both centers have collaborated for the creation and validation the model.

RESULTS: A decision tree was developed based on the accepted treatment alternatives from the NCCN guidelines 2015 and the possibility of uncertain events resulting from treatment decisions. The usefulness was measured in recurrence-free survival. The optimal treatment decision was to administer Radiation therapy regardless of the status of estrogen receptors. For the decision to give hormone therapy, the optimal decision is to administer HT if the percentage of cells staining positive for estrogen receptor was high and not administer if the percentage was low. We found that the decisions obtained with the model are consistent with current recommendations and with accepted scientific evidence. We also made the computer adaptation of the model for easy use in routine clinical practice. We collected data from 266 patients treated at the IVO in the last 17 years, with a mean follow-up of 75 months. The patients had an overall survival of 99.25 % with a recurrence -free survival of 86.8 % after a median follow up of 75 months.

CONCLUSIONS: In this study we developed a decision treatment model for patients with DCIS based on Bayesian Decision Theory. The results were consistent with scientific evidence and clinical practice. Finally, we performed the computer implementation of the model in order to be used in routine clinical practice.

DISCUSION: The model developed in the study is the first decision model that takes into account the uncertainty calculation based on the expected utility of each decision on recurrence-free survival for each patient.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-17-05

Title: Claudin-4 expression is associated with disease free survival in breast carcinoma in situ: Mean follow up of 8.2 years

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Body: Introduction: Claudins are tight junction molecules that have been associated with breast cancer prognosis. The claudin-low intrinsic subtype of invasive carcinoma was recently described and associated with high grade carcinoma, low junction molecules expression and worse response to chemotherapy. However, it is not known whether the expression of claudins may provide clues as to carcinoma in situ prognosis. The aim of study was evaluate the association between claudin-4 expression and disease-free survival and histologic type of local recurrence in carcinoma in situ after longer follow up.

Methods: A tissue microarray (TMA) block was constructed from 137 pure carcinoma in situ paraffin blocks sampled from patients treated from 1999 to 2009. The TMA was submitted to immunohistochemical staining for claudin-4. A claudin-4 score calculated based on percentage and intensity of expression, categorized samples as: claudin-4-low or claudin-4-high. Clinical data, treatment data, local recurrence data and survival of each patient were reanalyzed from medical records. Kaplan-Meier curves, log-rank and Wilcoxon tests were used to analyze disease-free survival; qui-square and Fisher test were employed to compare others variables; a significance level of 5 % was used.

Results: Claudin-4 expression was evaluated in 86 samples, 88.4% were claudin-4-high and 11.6% claudin-4-low. Mean follow up was 8.2 years (and local recurrence rate was 10.5 %. There was significant difference in the disease-free survival between claudin-4-high and claudin-4-low (4.9 x 1.9 respectively, $p= 0.02$); however there was no difference between both in histologic type of recurrence, invasive or in situ ($p=0.44$).

Conclusion: In our samples, claudin-4-high expression in carcinoma in situ was more frequent than low expression. Our data showed that claudin-4-low expression had a worse prognosis in carcinomas in situ (inferior disease-free survival) but it was similar to claudin-4-high in histologic type of local recurrence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-17-06

Title: LORIS trial of active monitoring for DCIS: How does the online pathology eligibility review process work?

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Body: Introduction

The LORIS Trial is a UK randomized clinical trial comparing active monitoring with surgery for low risk ductal carcinoma in situ (DCIS), defined as low or low-intermediate grade DCIS without comedo necrosis, as diagnosed on vacuum-assisted (wide bore) core needle samples. Because of the inconsistency of grading DCIS, we have underpinned this trial with a Central Histopathology Review (CHR) before randomisation. The process of the CHR for the first 22 months of a two year pilot study between July 2014 and May 2016 is reported here.

Patients and methods

Patients were eligible for CHR if they satisfied all of the eligibility criteria and had locally reported low or intermediate grade DCIS. Patients were identified at 28 pilot sites and were registered for potential trial entry following written informed consent before being subjected to CHR. CHR comprised online examination of digitally scanned histology slides of all material from all diagnostic biopsies and was performed by at least two of the three LORIS specialist breast pathologists. Histology slides were submitted using Royal Mail Safebox® to the University of Birmingham where they were digitally scanned and made available for review via the Leica digital image hub. The outcome of the review was reported in a separate secure online database by completion of a Central Pathology Review Form. Access to both online systems is password protected. Eligibility was confirmed if two pathologists agreed that there was low or low to intermediate grade DCIS and no comedo necrosis. A maximum of 7 calendar days from receipt of the diagnostic material was allowed for the central review process.

The digital images of the histology slides are stored by the Leica system for future reference.

Results

100 patients were registered and their slides reviewed. 55 of these were deemed eligible by CHR; of these 38 have been randomised. 45 patients were deemed ineligible, most commonly due to grade being in the upper half of the intermediate category and/or comedo necrosis. In addition, 9 patients were deemed not to have DCIS and 1 patient had invasive disease. Grouping the grade categories as low and low to intermediate grade (low risk and eligible for randomisation) Vs intermediate to high and high cytonuclear grade (ineligible for randomisation) showed 91% agreement on grade category amongst the reviewing pathologists.

Results of the central review were made available to sites within 7 days for 97% of cases submitted. On average, central review was completed within 4 days. Average time between registration and randomisation was 3 weeks. The LORIS central review pathologists found online viewing and reporting of sections acceptable.

Conclusions

Central Histopathology Review using online viewing of digital slides provides timely and efficient pathology Quality Assurance in this clinical trial setting, with acceptable turnaround times and good agreement between reviewing specialist breast pathologists. This process will be continued in the main phase of the trial.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-17-07

Title: Improved long-term outcomes of breast-conserving therapy for women with ductal carcinoma *in situ*

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Body: Purpose: Improved mammographic and surgical techniques and pathologic evaluation, particularly greater attention to achieving negative margins, have resulted in decreased local recurrence rates for patients with ductal carcinoma in situ (DCIS). This is an updated analysis of local outcomes after breast-conserving surgery (BCS) and adjuvant radiation therapy (RT) at a single institution in the modern era.

Methods and Materials: We retrospectively reviewed the records of 245 women treated for DCIS with BCS and RT between 2001 and 2007. Competing risk analysis was used to calculate local recurrence (LR) as a first event with the development of a second non-breast malignancy, contralateral breast cancer, and death as competing first events. The median age at diagnosis was 54 (range, 32-84) and 174 (93%) women had estrogen receptor (ER) and/or progesterone receptor (PR) positive disease. Ninety-five (39%) were grade III. Specimen radiograph during surgery was obtained for 223 women (91%) and post-operative mammogram for 102 (42%). Half underwent more than one excision. The institutional goal for margins during the study period was 3 mm or greater; final margins were >2 mm in 221 (90%). All received adjuvant radiation therapy to the whole breast (median whole breast dose: 4400; range, 4000 - 5220) and nearly all (99%) received a boost to the surgical cavity (median boost dose: 1600; range, 800 – 1800). Among patients with ER and/or PR+ disease, 105 (60%) received adjuvant hormonal therapy.

Results: At a median follow-up of 10.6 years, 4 patients had a LR (2 DCIS, 2 invasive ductal carcinoma) as a first event with a cumulative LR incidence of 0.0% and 1.5% at 5 and 10 years, respectively. The 5 and 10-year cumulative incidence of the competing first events is seen in the table below. Twenty women developed a contralateral breast cancer (CBC; 8 DCIS, 12 invasive carcinoma), 13 were diagnosed with a second non-breast malignancy (3 endometrial, 2 fallopian tube, 1 gallbladder, 1 leukemia and thyroid, 4 lung, 1 ovarian, and 1 uterine), and 7 died. Family history, age at diagnosis, and receipt of hormonal therapy were not significantly associated with the development of CBC on univariable analysis (all $p > 0.05$).

Incidence of local recurrence and competing events

Event	5-year cumulative incidence	10-year cumulative incidence
Local recurrence	0.0%	1.5%
Contralateral Breast Cancer	2.5%	7.9%
Second non-breast malignancy	2.6%	4.5%
Death	1.2%	3.5%

Conclusions: With longer follow-up, our rates of local recurrence following breast-conserving therapy for DCIS remain very low (1.5% at 10 years). The vast majority of patients had >2 mm margins, specimen radiographs, and received a tumor bed boost. The majority (60%) of patients with hormone receptor positive disease received adjuvant endocrine therapy. The 10-year incidence of CBC was higher than expected. Predisposing factors for the development of CBC are worthy of investigation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-17-08

Title: The role of sentinel lymph node biopsy in patients with ductal carcinoma in situ. An updated meta-analysis involving 9803 patients

Chehade HEH, Headon H, Wazir U, El Tokhy O, Kasem A and Mokbel K. London Breast Institute, London, United Kingdom.

Body: Background: Ductal carcinoma in situ (DCIS) is the predominant pre-invasive neoplasia of the breast. It was observed that omission of axillary dissection in those with pure DCIS had no adverse effect on survival or recurrence. Therefore, axillary dissection typically does not feature in the management of DCIS. However, it has recently been purported that in some cases of DCIS, the axillary lymph nodes may show evidence of invasive disease. Consequently, there may be a role for sentinel lymph node biopsy (SLNB) in patients with DCIS with a high risk of invasion.

Materials and Methods: Systematic literature review identified 48 studies (9803 DCIS patients who underwent SLNB). Separate analyses for patients diagnosed preoperatively by core sampling and patients diagnosed postoperatively by specimen pathology were conducted to determine the percentage of patients with axillary nodal involvement. Patient factors were analysed for associations with risk of nodal involvement.

Results: The mean percentage of positive SLNBs was higher in the pre-operative group (5.95% vs. 3.02%; $p=0.0201$). Meta-regression analysis showed a direct association with tumour size ($p=0.0333$) and grade ($p=0.00839$), but not median age nor tumour upstage rate.

Conclusions: SLNB should be considered in patients with a pre-operative diagnosis of extensive and/or high-grade DCIS after a careful multidisciplinary discussion in order to identify those patients who have unrecognised axillary spread.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-17-09

Title: Resolution of DCIS in patients with early breast cancer receiving primary chemotherapy for invasive breast cancer

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Body: Background

Ductal Carcinoma in Situ (DCIS) plays a pivotal role in surgical planning for patients who are to undertake primary chemotherapy. As DCIS is not thought to be responsive to chemotherapy, many women with large primaries or extensive DCIS on initial diagnosis will be planned for and undergo mastectomy. To investigate the role of chemotherapy in invasive breast cancer with concomitant ductal carcinoma in situ (DCIS), we examined patients who had primary systemic therapy for a primary invasive cancer with either radiologically or histologically proven DCIS to see if there were patients in whom there was no evidence of DCIS at resection.

Methods

This was a retrospective single centre study. Examining the records of all patients who had received primary chemotherapy between January 2010 and October 2014.

Patients were identified through the Guy's breast cancer database and chemotherapy prescribing system. To fully assess the DCIS status, all patients were cross-referenced with the electronic notes on our electronic noting system (MOSAIQ), radiology on Patient Archiving and Communication System (PACS) and histology on our Electronic Patient Record (EPR).

Results

1526 patients were identified, of whom 156 underwent primary chemotherapy. Of these, 46 patients had a pre-chemotherapy biopsy confirming DCIS, of whom 30 also had radiological evidence of DCIS. A further 26 had micro-calcification on their initial imaging which was presumed to be DCIS.

Twelve of the 46 patients with biopsy proven DCIS at presentation did not have DCIS at resection. Of these 9 had a mastectomy, with 5 achieving a pathological complete response (pCR), of whom 4 had a complete radiological response (rCR).

Of the 26 who had micro-calcification pre-chemotherapy, 15 did not have DCIS in the resection specimen. Of these, 10 had a mastectomy, with 6 having a pCR of whom 3 also had rCR.

Conclusion

This retrospective study suggests that chemotherapy can influence DCIS, with 12 biopsy proven having a pCR post treatment. This may indicate that some patients may be spared mastectomy.

Although there were patients with radiological evidence of DCIS, without a confirmatory biopsy we cannot be sure that these were malignant. This highlights the need to ensure that all suspicious areas distant from the primary tumour should be biopsied before treatment.

In conclusion, for patients who have an excellent clinical and radiological response, even in the presence of DCIS at presentation, more intensive evaluation is indicated if conservative surgery is a possibility.

2016 San Antonio Breast Cancer Symposium

Publication Number: P3-17-10

Title: The impact of the presence of ductal carcinoma in situ in patients with invasive ductal carcinoma

Xu L, Monga V, Thomas A and Leone JP P. University of Iowa Hospitals and Clinics, Iowa city, IA.

Body: Background: Ductal carcinoma in situ (DCIS) is considered to be a precancerous lesion that shares many genetic similarities with invasive ductal carcinoma (IDC). However, it remains unclear how DCIS might develop into IDC and whether the coexistence of DCIS has any clinical significance. There is limited data on whether coexisting DCIS in patients with IDC (IDC-DCIS) has any impact on patients' clinical presentation, tumor characteristics, prognosis and treatment selection. We aim to investigate differences in patients with pure IDC versus patients with IDC-DCIS.

Methods: We reviewed clinicopathologic data from the Breast Molecular Epidemiological Resource (BMER) database, which is a prospectively maintained breast cancer database from the University of Iowa. Missing information was supplemented by Iowa Cancer Registry database. Patients with a diagnosis of pure IDC and IDC-DCIS from 2009 to 2014 who underwent surgical resection of their breast cancer were included. We excluded patients with stage IV disease at diagnosis and those who underwent neoadjuvant therapy. Patients who had more than one tumor were only analyzed once using parameters of the largest tumor. Patient and tumor characteristics and treatment selection were compared between the IDC and IDC-DCIS groups. Student's t test was used for continuous variables and chi squared test for categorical variables.

Results: We observed that women with IDC-DCIS (n=226) had higher incidence of Her-2 positive cancers than those with pure IDC (n=95) (p=0.04). The IDC-DCIS group was more likely to be ER + and PR +, though these differences were not statistically significant. Another distinguishing characteristic between the two groups was that the IDC group contained more current smokers than the IDC-DCIS group (18.9% vs 10.6%, p<0.01). Patients with IDC-DCIS were more likely, than patients with pure IDC, to be under-staged based on clinical information. Clinical stage distribution in IDC-DCIS group was: 4% stage 0, 61.9% stage I, 28.3% stage II and 5.8% stage III. In contrast, the percentages of pathologic stage I, II and III were 54.5%, 35.4% and 10.2%, respectively (p=0.002). Similar analysis for patients with pure IDC showed no significant overall change from clinical to pathologic stage. Patients with IDC-DCIS tended to have higher total mastectomy rates than patients with IDC (37.2% vs 31.6%, p=0.34). Management of patients in either group were not significantly different in terms of radiation, chemotherapy, or hormonal therapy. There were 4 deaths (4.2%) in the IDC group and 12 deaths (5.3%) in the IDC-DCIS group (p=0.68).

Conclusions: Our study showed that active smoking may be a risk factor for the development of IDC without pre-existing DCIS. Patients with IDC-DCIS had higher rates of Her-2 positivity and significant differences between clinical and pathologic stages. Management and survival of both groups were similar.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD5-01

Title: Microenvironment microarrays show that microenvironment mediated resistance mechanisms to lapatinib differ between basal and luminal HER2+ cells

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Body: Cell lines represent a valuable model system for the study of breast cancer, as they capture the cellular diversity, mutational spectrum, expression subtypes, and genomic alterations that are observed in clinical specimens. However, like any model system, cell lines are imperfect, particularly when it comes to capturing the effects of the myriad of signals and interactions they encounter in their microenvironment (ME). We are utilizing a technology known as microenvironment microarrays (MEMA) to begin to unravel the consequences of interactions of breast cancer cells with the ME. MEMA consist of thousands of unique combinations of insoluble matrix proteins that are printed to form growth pads with ligands added to the media. Cells are grown on the MEMA spots and the effects of the specific ME that they are exposed to can be read out using immunofluorescent stains of interest. When combined with automated imaging and sophisticated image processing and analysis, the MEMA platform enables the identification of specific ME conditions that alter the phenotypes of cells. We have applied MEMA to understand both baseline responses to the ME as well as how the ME might mediate response to therapeutics. We performed a pilot experiment to investigate the effects of the ME on the response to the HER2-targeted inhibitor lapatinib. We found that HCC1954 cells continued to proliferate robustly in the presence of HGF when treated with 500 nM lapatinib. In contrast, AU565 cells were proliferative in the presence of NRG1 and lapatinib, but not HGF. Focused follow up studies showed that HGF is effective in rescuing only basal HER2+ cells, while NRG1 is effective in rescuing only luminal subtype HER2+ cells. Rescue with the relevant growth factor was also observed in 3-d matrigel studies, showing this was not an artifact of the 2-d culture system. We investigated the effects of drug combinations using lapatinib plus drugs that target either MET (Crizotinib) or HER3-HER2 dimers (pertuzumab). These drug combinations were able to overcome the resistance mediated by HGF and NRG1 in basal and luminal cells respectively. We found the effectiveness of pertuzumab particularly interesting, given that lapatinib should still be inhibiting HER2 kinase activity. Parallel studies found that inhibitors targeting other kinase receptors such as IGF1R partially restored sensitivity to HER2 in the presence of NRG1, suggesting a role for such receptors in the resistance. Immunoprecipitation studies showed that IGF1R co-immunoprecipitated with HER2/HER3 when pertuzumab was absent, but that additional of pertuzumab abrogated the binding of IGF1R to HER3, suggesting the formation of HER2-dependent higher order structures that can signal even when HER2 is inhibited. These studies highlight the importance of understanding the effects of the ME on cancer cells, and demonstrate the differences between ME factors that can confer resistance to HER2 targeted inhibitors in basal and luminal HER2+ cells. These findings suggest that both subtype and ME composition may be important in determining response to combinatorial treatments and may be useful to inform clinical decision making.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD5-02

Title: Paclitaxel induced mena- and TMEM-mediated pro-metastatic changes in the breast cancer microenvironment

Karagiannis GS S, Pastoriza JM M, Wang Y, Harney AS S, Entenberg D, Pignatelli J, Jones JG G, Anampa J, Sparano JA A, Rohan TE E, Condeelis JS S and Oktay MH H. Albert Einstein College of Medicine, Bronx, NY and Montefiore Medical Center, Bronx, NY.

Body: Background: Breast cancer cell intravasation and dissemination occurs specifically at microanatomical structures that we call tumor-microenvironment of metastasis (TMEM), representing direct physical contact between a tumor cell expressing the actin-regulatory protein Mammalian-enabled (Mena), a perivascular Tie2^{hi}/Vegf^{hi}-expressing macrophage, and an endothelial cell (Harney et al. Cancer Discovery 2015). TMEM sites have been identified in mouse and human mammary carcinomas, and both TMEM density (Rohan et al. JNCI 2014) and invasive Mena isoform expression (Agarwal et al. Breast Cancer Res, 2012; Forse et al. BMC Cancer, 2015) correlates with metastasis in early stage breast cancer. Since cytotoxic agents such as PTX induce influx of bone marrow-derived progenitors that differentiate into Tie2^{hi}/VEGF^{hi} macrophages in the primary tumor, we hypothesized that PTX may potentiate tumor cell invasion and metastasis by inducing the formation of TMEM sites and/or function.

Methods and Results in humans: We analyzed the effect of chemotherapy on TMEM and invasive Mena isoforms in 10 patients with localized breast cancer who had residual disease after neoadjuvant chemotherapy (NAC: weekly paclitaxel followed by dose-dense doxorubicin-cyclophosphamide [AC]), of whom 7 had more than 2-fold increase in TMEM density in residual disease compared with pretreatment. In a separate cohort of 5 patients, NAC produced an acute increase of up to 150-fold in invasive Mena isoforms after 1-2 doses of NAC.

Methods and Results in mice: After our preliminary data in humans, we evaluated effects of PTX in 4 different models, including 2 mouse models (PyMT-spontaneous & transplantation) and 2 patient-derived xenograft (PDX) triple negative models (HT17, HT33). Although PTX delayed primary tumor growth, tumors in PTX-treated mice had significantly more TMEM sites, circulating tumor cells (CTCs) and metastatic foci when compared to vehicle-treated animals. Using intravital imaging of MMTV-PyMT-Dendra2/Cfms-CFP mice, PTX induced influx of macrophages into primary tumors and intravasation of cancer cells at TMEM sites. Furthermore, PTX treatment significantly increased expression of Mena at the gene and protein levels, including invasive Mena isoforms. Deletion of the Mena gene completely abolished dissemination and metastasis in all cases, including those treated with PTX.

Conclusions: We show in mammary carcinoma mouse models and PDX models that although PTX delays tumor growth, it induces invasive Mena isoform expression and significantly increases the density of TMEM sites that are responsible for cancer cell intravasation, dissemination and metastasis. Thus, our data indicate that PTX paradoxically induces dissemination of breast cancer cells by promoting invasive Mena isoforms and TMEM-mediated cancer cell intravasation, suggesting that blockade of TMEM assembly and/or function could enhance the effectiveness of PTX and possibly other cytotoxic agents commonly used to treat early and advanced stage breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD5-03

Title: Effect of PD-1 and PD-L1 in the tumor microenvironment on overall survival of triple-negative breast cancer patients

Linnaus ME E, Kosiorek H, Barrett MT T, Dueck A, Anderson KS S, Ocal IT T, McCullough AE E, Annamalai L, Yearley JH H and Pockaj BA A. Mayo Clinic Arizona, Phoenix, AZ and Merck & Co, Inc., Kenilworth, NJ.

Body: Introduction: The programmed cell death-1 (PD-1) immune checkpoint is a normal regulator of the autoimmune response to inflammatory stimuli. Several cancers have taken advantage of this mechanism by harboring its ligand (PD-L1), thus activating the checkpoint to evade immune destruction. Increased expression of PD-L1 on tumor cells has been linked with worse outcomes in several cancers, including breast cancer. In triple-negative breast cancer (TNBC), expression of PD-L1 is increased compared to luminal cancers and as such, has become a favorable target for anti-tumor therapies. However, few studies evaluate the impact of PD-1 and PD-L1 expression on overall survival (OS) in TNBC. The aim of this study was to assess OS based on PD-1 and PD-L1 positivity and distribution in the tumor microenvironment. **Methods:** All patients with newly diagnosed TNBC from 1999 to 2015 with adequate pathologic specimen were identified for evaluation. Surgical pathology specimens were stained by immunohistochemistry (IHC) for PD-1 and PD-L1. Those considered PD-1+ or PD-L1+ had a staining score of 3, 4, or 5. Patient and clinical characteristics of the adjuvant group were compared by PD-1 and PD-L1 status with Chi-Square and ANOVA, as appropriate; characteristics of the neoadjuvant population were described. OS was calculated using Kaplan-Meier method and compared by Log Rank test for PD-1, PD-L1 positivity and tumor microenvironment status. **Results:** Fifty-two patients met inclusion; 38 had surgery prior to chemotherapy while 14 had neoadjuvant chemotherapy. There were 15 PD-1+ patients (39%) and 21 PD-L1+ (55%) patients in the treatment naïve cohort. No significant difference was found in the treatment naïve group between age, race, postmenopausal status, final stage, or pathologic characteristics (e.g. tumor size, mitotic rate, and grade) for all PD-1+ and PD-L1+ patients. Of the 21 PD-L1+ patients, staining was present in the tumor, tumor/non-tumor (mixed), and non-tumor microenvironment 42.9%, 33.3%, and 23.8% of the time, respectively ($p=0.008$). No significant difference was found in OS between PD-1+ and PD-1- patients ($p=0.509$) or PD-L1+ and PD-L1- patients ($p=0.240$). However, distribution of PD-L1 staining significantly associated with OS. Those demonstrating PD-L1 staining in the tumor had a significantly higher OS than those with PD-L1 staining in the non-tumor microenvironment ($p=0.008$). In the subset of PD-L1+ patients, those with staining in the tumor show a 10-year OS of 100% versus 60% for those with staining in non-tumor ($p=0.006$). In the neoadjuvant cohort, 14.3% of patients expressed PD-1 positivity, while 42.9% had PD-L1 positivity; the distribution of the PD-L1 staining in tumor 14%, mixed 29%, and non-tumor 50%. No difference in OS was found based on PD-1 or PD-L1 ($p=0.660$) positivity in the neoadjuvant cohort. **Conclusion:** A large number of TNBC stain for PD-1 and/or PD-L1. The implications of this are yet to be determined. In this small cohort, pattern of staining had a significant impact on overall survival in treatment naïve patients. This finding will need to be validated on a larger cohort but may have important implications for treatment by defining patients who may benefit by checkpoint inhibitor therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD5-04

Title: Perioperative use of NSAID analgesic ketorolac may suppress early relapses in breast cancer: Perhaps transient systemic inflammation plays a role

Retsky MW W, Demicheli R, Forget P, Hrushesky W and Baum M. Harvard TH Chan School of Public Health, Boston, MA; Milan National Cancer Institute, Milan, Italy; Université Catholique de Louvain, Brussels, Belgium; University of South Carolina, Columbia, SC and University College London, United Kingdom.

Body: A bimodal pattern of hazard of relapse among early stage breast cancer patients has been identified in multiple databases from US, Europe and Asia. We are studying these data to determine if this can lead to new ideas on how to prevent relapse in breast cancer. Using computer simulation and access to a very high quality database from Milan for patients treated with mastectomy only, we proposed that relapses within 3 years of surgery are stimulated somehow near the time of surgery. Most relapses in untreated breast cancer are in this early category. We could identify both angiogenesis of dormant micrometastatic deposits (particularly for premenopausal node positive patients) and activation of single dormant cells. Retrospective data from a Brussels anesthesiology group suggests an unexpected mechanism. Ketorolac, a common NSAID analgesic used perioperatively in surgery produced far superior disease-free survival in the first 5 years after surgery. The expected prominent early relapse events in months 9-18 are reduced 5-fold. Transient systemic inflammation accompanying surgery could facilitate angiogenesis of dormant micrometastases, proliferation of dormant single cells, and seeding of circulating cancer stem cells (perhaps in part released from bone marrow) resulting in early relapse and could have been effectively blocked by the perioperative anti-inflammatory agent. If this observation holds up to further scrutiny, it could mean that the simple use of this safe, inexpensive and effective anti-inflammatory agent at surgery might eliminate early relapses. This may reduce breast cancer mortality by 25 to 50% at low cost and toxicity. The best responding subgroup may be triple negative breast cancer. These data suggest transient systemic inflammation (identified by IL-6 in serum) is the precipitating factor. CTC and cells released during surgery in the presence of transient systemic inflammation could account for the single cell activation that was prevented by perioperative NSAID. There are a number of mechanisms that could explain how the perioperative NSAID prevents surgery induced angiogenesis. We are pursuing a clinical trial in Nigeria (high incidence of triple negative breast cancer and early onset). What we describe is driven by a host response and may be a general effect not limited to breast. Breast cancer is a disease that runs its course in over a decade but it seems that most relapses are the result of events that occur in the week after surgery.

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2016 San Antonio Breast Cancer Symposium

Publication Number: PD5-05

Title: Metabolic obesity, adipose inflammation and aromatase: Potential drivers of breast cancer risk in women with normal body mass index

Iyengar NM M, Brown KA A, Zhou XK K, Subbaramaiah K, Giri DD D, Gucalp A, Howe LR R, Zahid H, Bhardwaj P, Wendel NK K, Falcone DJ J, Morrow M, Wang H, Williams S, Pollak M, Hudis CA A and Dannenberg AJ J. Memorial Sloan Kettering Cancer Center (MSKCC), New York, NY; Weill Cornell Medical College, New York, NY; Hudson Institute of Medical Research, Clayton, Victoria, Australia and McGill University, Montreal, QC, Canada.

Body: Background: Elevated body mass index (BMI) is associated with increased risk of postmenopausal breast cancer, which may be partly attributable to an inflammation-aromatase axis. Most individuals with elevated BMI harbor white adipose tissue inflammation (WATi), defined by the presence of crown-like structures in the breast (CLS-B). CLS-B are composed of a dead/dying adipocyte surrounded by CD68+ macrophages. This inflammation is associated with activation of NF- κ B and elevated expression of aromatase, which could contribute to tumor development. Additionally, WATi correlates with several circulating changes, including hyperinsulinemia, which increase breast cancer risk. Although breast WATi correlates with rising BMI, it is also present in some normal BMI individuals. Beyond inherited germline syndromes, the etiology of breast cancer in individuals with normal BMI is not well understood. Here we examined the impact of breast WATi on breast aromatase expression and circulating factors in women with normal BMI.

Methods: Non-tumorous breast tissue and fasting blood were collected from 72 women with BMI < 25 kg/m² undergoing mastectomy at MSKCC. Breast inflammation was detected by the presence of CLS-B using CD68 immunohistochemistry. The primary objective was to determine if breast WATi in normal BMI individuals correlates with elevated aromatase levels in the breast, measured by qPCR, western blotting, immunofluorescence and enzyme activity. Secondary objectives included assessment of breast adipocyte size and circulating metabolic and inflammatory factors.

Results: Breast inflammation was present in 39% of women. Median BMI was 23.0 (range 18.4 to 24.9) in women with breast WATi versus 21.8 (range 17.3 to 24.6) in those without inflammation (P=0.04). Aromatase mRNA expression was positively correlated with WATi (CLS-B/cm²; P=0.002). Those with severe WATi had highest aromatase mRNA levels, compared to those with no or mild WATi (P=0.005). Aromatase protein, assessed by measuring adipose stromal cell-specific immunofluorescence or western blotting, and activity were also higher in CLS-B+ cases compared to CLS-B- (P<0.001). Breast WATi correlated with larger adipocytes (P=0.01) and higher circulating levels of C-reactive protein, leptin, insulin, and triglycerides (P<0.05). Insulin resistance, characterized by the homeostasis model (HOMA2-IR), correlated with breast WATi (P=0.004). Finally, leptin, a known inducer of aromatase and driver of cancer growth, correlated with higher breast aromatase levels (P=0.02) and larger adipocytes (P<0.01).

Conclusions: A metabolically unhealthy state occurs in women with inflamed breast adipose despite having a normal BMI. This subclinical inflammatory state is characterized by elevated aromatase in the breast, insulin resistance, and dyslipidemia. The presence of enlarged adipocytes in the breasts of normal BMI women with inflammation suggests a state of hyperadiposity which could not be predicted based on BMI alone. These findings indicate that normal BMI metabolic obesity may be associated with increased cancer risk. Our results suggest that objective measurements of adiposity rather than BMI may help to identify individuals at increased risk for disease.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD5-06

Title: Prognostic value of molecular tumor infiltrating lymphocyte (mTIL) signatures in HER2-positive breast cancer patients in N9831 and FinHer/FinXX trials

Chumsri S, Serie DJ J, Mashadi-Hosseini A, Tenner KS S, Lattia SL L, Moreno-Aspitia A, McLaughlin SA A, Nassar A, Warren S, Danaher P, Colon-Otero G, Lindman H, Joensuu H, Perez EA A and Thompson EA A. Jacoby Center for Breast Health, Mayo Clinic, Jacksonville, FL; Mayo Clinic, Jacksonville, FL; 3NanoString, Inc., Seattle, WA; Mayo Clinic, Rochester, MN; Helsinki University Hospital and University of Helsinki, Helsinki, Finland; Mayo Clinic, Jacksonville, FL and Mayo Clinic, Jacksonville, FL.

Body: Background: While previous study showed that the enrichment of immune-related gene expression was associated with outcome in HER2+ patients receiving sequential or concurrent trastuzumab (H), stromal tumor infiltrating lymphocytes (sTIL) have not been consistently shown to associate with outcome in this group of patients. Given that TIL scoring may be subjective, we analyzed molecular signatures of different subsets of tumor infiltrating immune cell populations, using NanoString™ gene expression data to assess molecular TIL (mTIL) signature enrichment and intrinsic subtype as a function of relapse-free survival (RFS).

Methods: NanoString™ technology was used to quantify mRNA in samples from 1,280 patients in N9831, 168 patients in FinHer, and 170 patients in FinXX. In N9831, patients in arm A were treated with chemotherapy alone (AC-T), arm B received chemotherapy followed by sequential H (AC-T-H), and arm C received H concurrently with chemotherapy (AC-TH). In the FinHer trial, H was given concurrently for 9 weeks and either 1 year or 9 weeks in FinXX trial. Cox proportional hazard ratio (HR) was used to determine the association of each gene signature with RFS. Different immune subset signatures, including CD45, B-cells, CD8 T-cells, cytotoxic-cells, and T-cells were analyzed using algorithms developed by NanoString.

Results: In N9831, CD45, cytotoxic-cell, and T-cell signatures were significantly associated with improved RFS in patients receiving chemotherapy alone and AC-T-H. However, none of the mTIL signatures were significantly associated with outcome in patients receiving AC-TH. Patients lacking CD45 enrichment had better outcome when H was given concurrently with chemotherapy. The 10-year Kaplan-Meier estimates for RFS in arm B patients with CD45 enrichment or no enrichment were 81.3% and 72.6%, respectively (HR 0.63 [95% CI, 0.42-0.93]; $p = 0.02$), and in arm C were 83.6% and 79.8%, respectively (HR 0.79, 95%CI 0.49-1.28; $p = 0.34$). Among patients with HER2-enriched subtype, all of the mTIL signatures were associated with improved RFS in arm A (AC-T) and B (AC-T-H) but remained non-significant in arm C (AC-TH). In patients with luminal subtypes, mTIL signatures were not significantly associated with outcome in patients treated with chemotherapy alone. Similar findings were observed in the FinHer and FinXX trials, in which, none of mTIL signatures were significantly associated with outcome among patients who received H.

Conclusion: This analysis sheds light on previous discrepancy between immune-related gene signature and sTIL findings. Our data also suggests that the poor prognosis associated with lack of infiltrating immune cells can be partly overcome by the concomitant administration of H with chemotherapy. mTIL signatures, specifically CD45, cytotoxic, and T cells, were prognostically associated with improved outcome in patients receiving chemotherapy without concurrent trastuzumab. Understanding the role of the immune system in response to H will require a higher degree of granularity than can be achieved by histological quantification of TILs. Further studies are needed to validate the significance of mTIL signatures as predictive or prognostic biomarker in HER+ patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD5-07

Title: From monocyte re-programming to the expansion of tumor infiltrating lymphocytes for adoptive T cell therapy

Doucey M-A, Crespo I, Bou Mjahed R, Turrini R, Guex N, Ifiticene-Treboux A, Faes-van't Hull E, Ibberson M, Liechti R, Bron S, Lehr H-A, Delaloye J-F, Xenarios I and Coukos G. Ludwig Institute for Cancer Research, Lausanne Branch, Epalinges, Switzerland; Vital-IT Systems Biology Division, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland; Centre du Sein, CHUV (Centre Hospitalier Universitaire Vaudois), Lausanne and Institut für Pathologie im Medizin Campus Bodensee, Friedrichshafe.

Body: Tumor associated monocytes and macrophages are highly plastic cells reported to support cancer progression by promoting tumor angiogenesis and metastasis in patients as well as in a variety of experimental mouse models. Conversely, other studies have demonstrated the ability of tumoricidal monocytes to specifically destroy tumor cells. These contrasting biological activities, tumor development versus destruction, are mediated by macrophages with distinct functional polarization, ultimately dictated by microenvironmental cues. While much effort has focused on strategies to block the recruitment of circulating monocytes in tumor tissue, or in the depletion of tumor macrophages in vivo in tumor-bearing mice, newly emerging approaches designed to 'reprogram' tumor macrophages with anti-tumor properties are demonstrating promise for cancer immunotherapy. We show that tumor-associated TIE-2-expressing monocytes (TEM) are highly proangiogenic and immune suppressive cells critical for breast tumor vascularization and growth. By merging computational systems modeling and experimental approaches, we have uncovered treatments reprogramming pro-tumoral monocytes into immunologically potent cells capable of mediating an anti-tumor immune response. We report here that proangiogenic and suppressive functions of TEMs are similarly driven by TIE-2 and VEGF receptor tyrosine kinase and their ligands PlGF and Ang-2. These unraveled pathways and ligands, which underlie monocyte pro-angiogenic activity, have a strong predictive value for breast cancer patient relapse-free survival. Further, blocking of TIE-2 and VEGFR kinase activity induced TEMs reprogramming into cells with features of myeloid dendritic cells able to enhance anti-tumor T cell responses. Most importantly, this treatment when applied to the tumor microenvironment, allows for the first time the expansion in vitro of Tumor Infiltrating Lymphocytes (TILs) to numbers compatible with Adoptive Cell Therapy (ACT). ACT consists of the administration to cancer patients of autologous TILs expanded in vitro from patient tumors in large numbers and has proven to be exceptionally effective in metastatic melanoma. However, ACT is of limited clinical efficacy in various other cancer types including breast cancer and to date, to the best of our knowledge, no expansion of functional TIL has been reported for ACT purposes in breast cancer. We show here that the expanded TILs displayed mainly an effector memory phenotype, are specific for tumor antigens and kill autologous tumor cells in vitro in a MHC-dependent manner. Thus, this uncovered treatment combination represents the first intervention on the breast tumor microenvironment which overcomes TILs dysfunctions and provides for the first time the basis for ACT in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD5-08

Title: Expression of LAG-3 in breast cancer, and its association with subtype and outcome

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Body: Aim: To investigate the expression and clinical value of the immune checkpoint marker LAG-3 in breast cancer patients

Background: Lymphocyte-activation gene 3 (LAG-3) is a recently discovered immune checkpoint biomarker that is targeted by agents currently being evaluated in early phase clinical trials. LAG-3 functions as a cell surface receptor expressed following T cell activation and negatively impacts T cell functions. This biomarker has not yet been evaluated in large series of breast cancers with long term treatment and outcome data, in the context of subtype and other immune biomarkers.

Methods: Two tissue microarray series (a training set with N=330 and a validation set with N = 2203 patients) were constructed from breast carcinoma primary excision specimens from University of British Columbia hospitals, linked to detailed clinical and pathological data. None of these patients had received neoadjuvant treatment. 4µm sections were stained with an antibody to LAG-3 (clone 17B4) by immunohistochemistry using a Ventana Discovery Ultra automated slide stainer. LAG-3+ stromal and intra-epithelial tumor infiltrating lymphocytes (TILs) were reported as absolute counts per tissue microarray core. Stromal TILs (sTIL) were defined as lymphocytes present in the stroma not in direct contact with tumor nest whereas intra-epithelial TIL (iTIL) were lymphocytes in direct contact with carcinoma cells. All descriptive and survival analyses were conducted using SPSS software.

Results: LAG-3+ sTILs were found in 16% of breast cancer cases in both the training set and the validation set; LAG-3+iTILs were present in 14 and 11%, respectively. In both the training set and the validation set, the presence of LAG-3 (iTILs or sTILs) was significantly ($p<0.001$) associated with high grade tumors, estrogen and progesterone receptor negativity, high Ki67 index and with the HER2+ and basal-like subtypes. In survival analyses of ER negative patients, in both sets patients with LAG-3 T cells (iTILs or sTILs) had a significantly improved disease-specific survival ($p<0.05$). As with other lymphocyte biomarkers, this association was not observed among ER+ patients.

Conclusions: LAG-3+TILs are present in breast cancer and are associated with major risk factors and hormone receptor negative subtypes. ER negative breast cancer patients have a better outcome if they contain LAG-3+ TILs, consistent with published data showing better survival among ER- breast cancer patients with immune infiltrates. More than a quarter of ER negative breast cancers contain TILs expressing LAG3, and may represent the most relevant subset to target with emerging checkpoint inhibitors targeting this T cell surface receptor.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD6-01

Title: Analysis of breast cancer in young women in the department of defense (DOD) database

Zimmer AS S, Gatti-Mays M, Soltani S, Lipkowitz S, Steeg PS S, Zhu K, Perkins JG G, Hu H, Shao S, Brown D and Shriver CD D. Women's Malignancies Branch National Cancer Institute, NIH, Bethesda, MD; Murtha Cancer Center, WRNMMC, Bethesda, MD; Murtha Cancer Center, Walter Reed National Military Medical Center, Bethesda, MD and CSS Institute of Molecular Medicine at Windber, Windber, PA.

Body: Background: Women under the age of 40 account for approximately 7% percent of breast cancer patients. Breast tumors from young women are often ER-negative, occur in African-American patients, and have other indicators of high risk: yet, multivariate analyses demonstrated that young age is an independent predictor of poor outcome. Due to the unique nature of the patient population served by DOD, a disproportionate number of breast cancer cases in young women are seen. We compare the characteristics, treatment, and outcomes of young patients diagnosed with breast cancer with those of older patients.

Methods: The databases of the Military Health System Repository and the DOD Central Registration were used to identify female breast cancer patients treated at DOD facilities between 1998 and 2007. Information on demographics, breast cancer stage at diagnosis, definitive surgical treatments, systemic treatment, recurrence rate and overall survival was analyzed by age groups at the time of diagnosis (less than 40 years old, 40 to 49 years, and 50 years or older) using X² testing with significance defined as $p < 0.05$.

Results: We identified 10,066 women who were diagnosed with invasive breast cancer at DOD facilities between 1998 and 2007, of which 11.3% (1139) were less than 40 years old at diagnosis. 53% of this young cohort were white, 25% were African-American and 8% were Hispanic (14% undisclosed). The percentage of breast cancer among African-American women in the young cohort was higher than in the older cohorts (19.3% in 40-49yo and 10.6% in ≥ 50 yo). High-grade tumors were significantly more frequent in the younger cohort when compared to the older group (49.5% vs 34.7% and 25.2%, $p < 0.001$). < 40 yo most commonly presented with Stage II disease (45.3%) at diagnosis, while older groups were mostly diagnosed with Stage I disease (41.6% and 52.4%). The most common subtype of breast cancer across ages was ER+ disease, however, < 40 yo group had proportionally less ER+ (49% vs 61% and 67.3%, $P < 0.001$). There was a higher rate of bilateral mastectomies among the young women (18.4% vs 9.1% and 5.0%, $p < 0.0001$). Independently of the stage of disease, chemotherapy was given significantly more frequently to < 40 y (90.43%) and 40-49yo (81.44%) than ≥ 50 yo (53.71%). The 10-year overall survival of younger women was similar to the ≥ 50 yo cohort, despite intensive treatment.

Discussion: This study is one of the largest retrospective studies of women under 40 years old with breast cancer. Younger women with invasive breast cancer had more aggressive tumors presenting at higher stages. In this group with good access to healthcare, younger women still had a similar overall survival rate to older women despite receiving more aggressive treatment and potentially having fewer comorbidities than the older group.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD6-02

Title: Local recurrence in young women with invasive breast cancer; the prospective study of outcomes in sporadic and hereditary breast cancer (POSH)

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Body: Aim

To evaluate the surgical and clinical factors affecting local recurrence and other outcomes in young breast cancer patients using the POSH study.

Background

POSH (MREC:00/06/69) is a prospective cohort study of 3024 women aged 18-40 with breast cancer. Randomized controlled trials suggest equivalent survival between mastectomy and breast conserving surgery (BCS); however, very few young patients were included in these analyses. Emerging data also suggests a possible survival advantage for mastectomy in gene mutation carriers, and young age may be a predictor of higher rates of local recurrence.

Methods

Summary statistics were used to describe the cohort by surgical type (mastectomy or BCS). Study endpoints were in-breast ipsilateral local-recurrence interval, distant disease-free interval and overall survival. Cumulative hazard and Kaplan-Meier plots were used to describe survival data. Univariable and multivariable analyses were carried out using Cox proportional hazards models and flexible parametric survival (FPS) models (for any models with time-varying covariates).

Results

Of 2882 patients analysed, 1464 underwent mastectomy, 1395 BCS; and 23 had lymph node surgery only. Patients undergoing mastectomy had significantly larger tumours and higher proportions of positive Family History, positive estrogen receptor (ER+), progesterone receptor (PR+) and/or human epidermal growth factor 2 (HER2+) tumours. Local events only accounted for 15% of recurrences. Local recurrence varied over time by surgical type; local recurrence rate was similar at 18 months but higher for BCS compared to mastectomy at 5- and 10-years (18-months: 1.0% vs 1.0%, FPS-HR[95%CI]=1.43[0.89,2.32], $p=0.348$; 5-years: 5.3% vs 2.6%, FPS-HR[CI]=3.39[2.03,5.66], $p<0.001$; and 10-years: 11.7% vs 4.9%, FPS-HR[CI]=5.27[2.43,11.43], $p<0.001$, respectively). Similar results were found in the adjusted model and when restricting the analyses to patients with smaller tumours. Conversely, distant-metastases and death events were significantly lower for BCS but not after adjusting for prognostic factors. Chest-wall radiotherapy following mastectomy was associated with improved local recurrence interval (HR[CI]=0.46[0.24,0.86], $p=0.015$). Positive surgical margins, and the development of a local event, predicted for reduced distant disease free interval (HR[CI]=0.50[0.35,0.71], $p<0.001$; and 10-year FPS-HR[CI]=0.29[0.14,0.62], $p=0.001$, respectively).

Conclusions

In the short term, there is no difference in local recurrence between BCS and mastectomy in young women. Longer term, local recurrence is higher in BCS, but there is no difference in survival between surgical groups after adjusting for known prognostic factors. Surgical extent appears to be less important for distant relapse or death from breast cancer than completeness of excision or, where appropriate, chest-wall radiotherapy.

Acknowledgements

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2016 San Antonio Breast Cancer Symposium

Publication Number: PD6-03

Title: Preserving fertility in young women undergoing chemotherapy for early breast cancer; the Maastricht experience

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Body: *Purpose*

This study aimed to evaluate the uptake of fertility preservation, rate of pregnancy, pregnancy outcome and breast cancer outcome after diagnosis of early breast cancer in young women who were referred to Maastricht University Medical Center, from the regional hospitals in the Southeast part of the Netherlands.

Patients and methods

We prospectively registered the demographics of patients, who visited our university hospital for counseling on fertility preservation at diagnosis of stage I-III invasive breast cancer in the years 2008-2015. At baseline, tumor and treatment characteristics were registered. During follow-up information on fulfilled childwish and disease status was collected. To compare the fertility preservation group and the non-fertility preservation group independent samples Student *t*-tests and Chi-square tests were conducted.

Results

In total 128 women with a median age of 32 years (19 – 43) were referred for fertility preservation counseling before start of chemotherapy, with an increase in referral in the more recent years. Thirty-nine (30.5%) women chose for fertility preservation: in 26 patients embryos were frozen, in seven oocytes, and in one both embryos and oocytes. In four patients the procedure was not succesfull. Patients who had chosen for fertility preservation more often had a male partner (87.2% vs 70.8%, $P = 0.05$) and had a smaller tumor size (median 19 versus 23 mm, $P = 0.04$) at the time of diagnosis compared to those who did not chose for fertility preservation. During a median follow-up of 30.3 months (range 0 – 96.9), 27 (21.1%) patients had tried to conceive: 14 (35.9%) women in the fertility versus 13 (14.6%) in the non-fertility preservation group. All of these had recovery of ovarian function after chemotherapy-induced ovarian failure. Only two women used the cryopreserved embryos, both succesfully and combined with preimplantation genetic diagnosis of the embryos because of germline mutations in BRCA1-gene. Eight patients in the fertility preservation group and seven patients in the non-fertility preservation group became at least once pregnant. In the fertility preservation group, eight healthy babies were born, one baby had Morbus Hirschsprung, one women is pregnant at this moment and one woman had a miscarriage. Of the eleven pregnancies in the non-fertility preservation group, nine healthy babies were born and one woman had two miscarriages. Of the referred 128 women, nine (7.0%) had breast cancer recurrence, three in the fertility preservation group versus six in the non-fertility preservation group.

Conclusion

One third of referred patients choose for fertility preservation before start of chemotherapy. In all of these patients, the ovarian function recovered. However, two couples used their cryopreserved embryos for preimplantation genetic diagnosis and both became pregnant. Since the follow-up time is relatively short, more data are mandatory to make a statement on the effectiveness of fertility preservation techniques in young breast cancers patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD6-04

Title: HOHO study: How European and US young women cope with breast cancer and fertility concerns

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Body: Background

The International Breast Cancer Study Group (IBCSG), in collaboration with Dana-Farber Cancer Institute, conducts the European (EU) cohort of the Helping Ourselves Helping Others (HOHO) study. HOHO is a prospective longitudinal cohort study investigating short and long-term treatment and fertility concerns in young women with breast cancer (BC): most previous studies have been retrospective.

Patients and methods

From Aug 2009-Jan 2016, 300 patients aged 18-40 yrs with early/advanced BC were enrolled within 6 mos of diagnosis in 18 Centers in Italy and Switzerland. Patients are surveyed every 6 mos the first 3 yrs and yearly for additional 7 yrs. 297 baseline surveys were evaluable. The baseline survey includes sociodemographic and treatment data, and a modified Fertility Issues Survey (including fertility concerns, preservation, outcome and impact on treatment decisions). Clinical data are collected yearly. We qualitatively compare the EU baseline data with the published US data (Ruddy et al, JCO 32:1151, 2014).

Results

Almost two thirds of women in both cohorts discussed future fertility with their doctors at diagnosis. Fertility concerns, desire for future pregnancy (both before disease onset and at time of survey) and steps to reduce infertility were all more common in the EU cohort. EU women were also more concerned about fertility when discussing treatment and about a possible relapse affecting their ability to care for future children.

Conclusion

Many young women with newly diagnosed BC have concerns about fertility, which may affect their treatment decisions. In the EU cohort fewer women had a stable relationship and children before diagnosis, potentially influencing their treatment decisions and encouraging fertility preservation. Concerns about a possible relapse could partly explain the decrease in their pregnancy desire after the disease. Differences between EU and US women seem to emerge: continued data collection will determine if they persist over time. The POSITIVE Trial (IBCSG 48-14/ALLIANCE 221405) will address safety and outcome of pregnancy after BC.

	US	EU
	N (%)	N (%)
	620 (100)	297 (100)
Age <35 yrs	229 (37)	95 (32)
Married or in a significant relationship	474 (76)	192 (65)

Had children before BC diagnosis	409 (66)	160 (54)
Never pregnant	158 (25)	119 (40)
Before BC wished to have future children	230 (37)	161 (54)
At time of survey wished to have future children	160 (26)	120 (40)
If wanted more children, concerned about: (some women indicated more than one)	<i>n=160</i>	<i>n=120</i>
Caring for them if BC recurred	27 (17)	45 (38)
Children having increased risk of developing cancer	70 (44)	38 (32)
Pregnancy would increase risk of recurrence	-	56 (47)
Reason(s) for not wanting more children: (some women indicated more than one)	<i>n=460</i>	<i>n=177</i>
Caring for them if BC recurred	18 (4)	28 (16)
Children having increased risk of developing cancer	33 (7)	22 (12)
Pregnancy would increase risk of recurrence	59 (13)	16 (9)
Concerned about fertility at time of treatment decision-making (a little, somewhat or a lot)	319 (51)	189 (64)
Fertility concerns affected treatment decision (a little, somewhat or a lot)	160 (26)	115 (39)
Took special steps to decrease infertility	65 (10)	79 (27)
Discussed fertility issues with physician before starting therapy	424 (68)	198 (67)

2016 San Antonio Breast Cancer Symposium

Publication Number: PD6-05

Title: A prospective evaluation of clinical outcomes in women with pregnancy-associated breast cancer (PABC)

Jerzak KJ J, Zhu S, Li N, Mandel R and Warner E. Sunnybrook Odette Cancer Centre, Toronto, ON, Canada.

Body: Background:

Many studies suggest that women with PABC- breast cancer (BC) diagnosed during pregnancy or within 12 months post partum- have adverse outcomes compared to age matched women whose BC is not associated with pregnancy (non-PABC). However, it is unclear whether this is due to diagnostic delay alone or biological differences. Hence, we investigated whether PABC is independently prognostic for disease-free survival (DFS) and overall survival (OS) in a prospective database of young BC patients.

Methods:

A prospective database of women ≤ 40 years of age diagnosed with BC between February 2008 and January 2015 was analyzed. Data regarding, stage at diagnosis, pathology, treatment, and clinical outcomes were available. Statistically significant differences in baseline characteristics and administered therapies in women with and without PABC were evaluated using the chi-square or Fisher's Exact tests. Kaplan-Meier curves for DFS and OS in the PABC and non-PABC cohorts were compared using the log-rank test. A multivariate Cox proportional hazards model adjusted for age, nodal involvement and tumor size.

Results:

Of 224 women in the database who provided consent for research, 32 (12%) had PABC. Mean age of the PABC and non-PABC patients respectively was 34 (range 27 to 39) and 37 (range 21 to 40) and the median follow-up was 40 months in both groups. PABC was more likely to be locally advanced at diagnosis (44% vs. 22%, $p < 0.01$) and less likely to be hormone receptor positive (75% versus 85%; $p < 0.01$). There was no significant difference in age at diagnosis, tumor grade, lymphovascular invasion, HER2 expression or administered treatments between the two groups. Among the 166 women with early stage BC (not locally advanced), PABC was associated with positive lymph node status in a univariate model [OR 3.2 (95%CI 1.2-8.4), $p = 0.02$] but just missed significance in a multivariate analysis that adjusted for age and tumor size ($p = 0.06$).

Eight patients (22%) in the PABC group and 19 (10%) in the non-PABC group experienced local or distant disease recurrence; 3 patients (8%) in the PABC group and 11 (6%) in the non-PABC group died. The 3-year DFS in the PABC and non-PABC cohorts was 79% vs. 90% ($p = 0.22$) and the 3-year OS was 97% in both groups.

Conclusion:

Diagnostic delay could account for the higher rate of locally advanced disease in the PABC group. However, the lower hormone receptor expression and strong trend toward greater lymph node involvement independent of size suggest that women with PABC may have intrinsically worse disease biology. Event rates may still be too low to detect a statistically significant difference in recurrence risk. Further research is necessary to identify unique molecular features of PABC that may be amenable to targeting.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD6-06

Title: Oncologic outcome of pregnancy associated breast cancer: A case-control study

McCartan D, Kamer S, Eaton A, Seidman A, Murphy C, Gemignani M and Goldfarb S. Memorial Sloan Kettering Cancer Institute, New York, NY.

Body: Introduction and Aims

Pregnancy-associated breast cancer (PABC) is defined as the diagnosis of invasive breast cancer during the gestational period, within one year of pregnancy or any time during lactation. A diagnosis of PABC has traditionally been attributed a poor prognosis. The aim of this study was to assess the long-term outcome of patients diagnosed with PABC compared to a cohort of age-matched control patients.

Methods

A single-institution retrospective chart review was performed in 188 patients with PABC treated between the years of 1992 and 2015. Non-PABC controls were selected to match based on age and year of diagnosis. Clinicopathologic features, surgical and adjuvant treatments received, and clinical outcomes were assessed. Patients who were stage IV at diagnosis were excluded. Overall survival was estimated using Kaplan-Meier methods and compared between cases and controls using a log-rank test stratified on matched pair.

Results

Out of 188 patients with PABC, 63 (34%) were pregnant at the time of diagnosis and 125 diagnosed within 1 year of pregnancy (average: 6 months post partum). The characteristics of the PABC and case-matched controls are listed in Table 1

Table 1: Demographics, treatment, and outcome of PABC and non-PABC case-matched controls

		PABC n=188	Non PABC n=188	p-value
Demographics	Age (mean)	35.7yrs	35.9yrs	0.633
	BRCA1/2 carrier	22%	12%	0.072
Surgery	Breast Conservation	22%	38%	0.001
	Immediate reconstruction post mastectomy	81%	84%	0.544
Pathology	Grade 3	88%	70%	<0.001
	ER and/or PR positive	56%	72%	0.003
	HER2 positive	27%	22%	0.283
	Triple negative	27%	20%	0.145
	Node positive	27%	47%	0.039
Clinical Stage	I-II	67%	79%	0.010
	III	33%	21%	
Adjuvant treatments	Chemotherapy	99%	21%	<0.001
	Neoadjuvant	18%	17%	0.849
	Anthracycline	84%	81%	0.393
	Taxane	82%	75%	0.148
	Anti-HER2	20%	15%	0.238
	Anti-estrogen	53%	68%	0.003
	Radiotherapy	57%	62%	0.400
5-year OS		88%	95%	0.746

Figures are reported as percentages for categorical variables. OS: Overall survival

. Important pathological differences were evident with PABC patients compared to the non-PABC controls. PABC cases were more likely to be high grade ($p < .001$), node positive ($p = .039$) and less likely to be estrogen or progesterone receptor positive ($p = .003$). The majority of both cases and controls received chemotherapy (18% neoadjuvant). In the patients who were pregnant at time of diagnosis, 19 received chemotherapy during their pregnancy, with the most common combination being AC followed by paclitaxel in the post-partum period. Five year overall survival was similar for both groups, 88% for PABC patients and 95% for non-PABC case controls ($p = .746$) with a median follow up of 5.1 years.

Conclusion

Diagnostic and therapeutic advances have improved the oncological outcome for breast cancer patients, but not all of these have been adapted for pregnant patients. The consistent finding of higher-stage tumors in these patients reinforces the importance of utilizing developments in systemic treatments, potentially through case registries, to evaluate outcomes. PABC is associated with more adverse features than non-PABC controls matched for age and year of diagnosis. However, PABC is not in itself an adverse prognostic factor for survival after correcting for pathologic features.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD6-07

Title: PD-L1 is highly expressed in tumor infiltrating lymphocytes in pregnancy associated breast cancer

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Body: Background: Pregnancy associated breast cancer (PABC), diagnosed during or after gestation, is typically triple negative, and is associated with a poor prognosis. Those diagnosed within two years have an even worse outcome. We previously assessed the immune microenvironment of invasive breast carcinomas in young women and reported that tumor infiltrating lymphocytes (TILs) were more prominent in PABC. Programmed cell death protein 1 (PD-1) is upregulated following activation of lymphocytes, while programmed death ligand 1 (PD-L1) is one of the primary ligands that it interacts with to inhibit T-cell activation and proliferation. PD-L1 may also be constitutively expressed on tumor cells as a result of oncogenic signaling or epithelial-mesenchymal transition. Emerging evidence suggests that the effect of the local immune system, particularly the interactions between PD-1 and PD-L1, is also key in breast cancer progression and in breast tumor responses to chemotherapy and targeted therapy. In this study, we assessed expression of PD-1 and PD-L1 in both TILs and tumor cells in PABC and in age-/stage-/grade-matched nulliparous women, and correlated their expression with clinicopathologic characteristics in this aggressive type of breast carcinomas.

Design: 21 patients diagnosed with PABC within two years of pregnancy (mean age=35.7, range=26-48) and 15 matched controls (mean age=37.5, range=29-51) were evaluated. Slides were reviewed and pathologic tumor characteristics, including TILs, were noted. Immunohistochemical stains for PD-1 and PD-L1 were performed. Extent (1=1-25% positive tumor cells, 2=26-50%, 3=51-75%, 4=76-100%) and intensity (1=weak, 2=moderate or 3=strong) of staining were assessed. A composite score (CS) was calculated by multiplying the extent by intensity (range=0-12; weak=1-3; moderate=4-8 and strong=9-12).

Results: The mean CS for PD-L1 in TILs was significantly higher in PABC (5.86) compared to controls (3.07), $p=0.03$. Further, strong expression of PD-L1 in TILs was only observed in PABC (9/21, 42.9%); none of the controls had strong PD-L1, $p=0.01$. The high expression of PD-L1 in PABC TILs was independent of tumor grade, hormone receptor and HER2 status, and other histologic features including lymph node metastasis. Expression of PD-1 in TILs was similar in both PABC and controls (mean CS 6.81 and 5.36, respectively). Immunoreactivity in the tumor cells themselves was rare with only two PABC and four control cases expressing PD-1 and PD-L1.

Conclusion: 1. TILs in PABC have significantly higher PD-L1 expression. 2. Strong expression of PD-L1 in TILs was only observed in PABC. 3. High PD-L1 expression in TILs was independent of the tumor characteristics in this series. 4. PD-1 is expressed similarly in TILs in both PABC and controls. 5. Rare cases may have PD-1 and PD-L1 expression in the tumor cells themselves. The results of our study showing significant expression of PD-L1 in PABC TILs add to the understanding of the role of the microenvironment in breast cancer progression. These complex interactions between tumors cells and the local immune system may predict response to therapy and investigation into the role of immune based therapies is under way in these aggressive breast carcinomas that affect young women.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-01-01

Title: Phase II in-human dose escalation study of the optical molecular imaging tracer bevacizumab-800cw for molecular fluorescence guided surgery in primary breast cancer patients

van Dam GM M, Koller M, Qiu SQ Q, Linssen MD D, de Vries J, Jansen L, Kelder W, de Jong JS S, Jorritsma-Smit A, van der Vegt B, Robinson DJ J and Nagengast WB B. University Medical Center Groningen (UMCG), Groningen, Netherlands; Martiniziekenhuis, Groningen, Netherlands; University Medical Center Utrecht, Utrecht, Netherlands and Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands.

Body: Introduction

Molecular Fluorescence Guided Surgery (MFGS) might be used for intraoperative detection of positive resection margins in breast conserving surgery (BCS) for the treatment of breast cancer. Currently, the presence of tumor positive resection margins can only be assessed *ex vivo* by histopathological analysis of the excised tissue, which takes up to 5 working days. The current study defined the optimal dose of the near-infrared (NIR) optical imaging tracer bevacizumab-800CW to enable intraoperative detection and image-guided pathology of tumor positive resection margins in breast cancer patients.

Methods

Molecular Fluorescence Guided Surgery during BCS was performed in subjects treated for primary breast cancer. The NIR optical imaging tracer bevacizumab-800CW targeting vascular endothelial growth factor A was administered intravenously three days prior to surgery in four escalating dose groups (4.5mg, 10mg, 25mg and 50mg). NIR fluorescent signals were detected real-time using an intraoperative fluorescence camera system (SurgVision BV). Standardized *ex vivo* analyses of tumor-to-normal ratios (TNR) were performed to define the optimal tracer dose using a BlackBox imaging system (SurgVision BV) for imaging fresh bread loaf slices, a NIR fluorescence flatbed scanner (Odyssey, Li-Cor) for imaging 10 μ m slices of formalin-fixed paraffin-embedded (FFPE) blocks, NIR Confocal Laser Endomicroscopy (CLE, Mauna Kea Technologies) and multi-diameter single fiber reflectance and single fiber fluorescence (MDSFR/SFF) spectroscopy quantification to enable correction for the influence of tissue optical properties on fluorescence in tumor and normal breast tissue.

Results

Currently, 12 subjects have been included and analyzed in four dosing groups. All tumors showed specific tracer uptake at macroscopic and microscopic level during *ex vivo* analyses, confirmed by histopathology. Quantification of NIR fluorescent signals showed higher TNRs by increasing doses up to 25mg. No further increase in TNR was seen in the 50mg dose group. CLE showed the feasibility of visualization of the tracer accumulation in tumor tissue compared to normal tissue at a microscopic level. MDSFR/SFF spectroscopy objectively confirmed the dose dependency up to 25mg.

Conclusion and future perspective

Intravenous administration of bevacizumab-800CW in doses up to 50mg is safe and highly tumor specific, showing a plateau of TNR at 25mg and 50mg. Further expansion of the dosing cohorts of 10mg and 25mg with additional seven patients per group will be performed to establish the optimal dose for MFGS during BCS for an upcoming phase III multicenter randomized controlled trial. By enabling MFGS during BCS the surgical treatment of primary breast cancer patients might be optimized by a reduced need for re-excision surgery and thereby reducing the risk of co-morbidity, psychological burden, poor cosmesis and healthcare costs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-01-02

Title: Diagnostic value of breast specific gamma imaging for breast cancer

Mallik DK K, Zhang H, Shi H, Tan H, Shen L, Hu G, Hu H, Sejdinaj F, Pang Y, Zhu W and Yang W. Zhongshan Hospital, Fudan University, Xuhui District, Shanghai, China.

Body: In this study, 422 female patients who presented with clinical indications and underwent ultrasound (US), mammography (MMG), and breast specific gamma imaging (BSGI) before surgery during the period from July 2013 to June 2015 were retrospectively reviewed. Twenty-two of the patients had no pathological report available and were excluded. These patients who presented with clinical indications underwent both ultrasound and mammographic examinations initially. If both US and MMG were positive, the patient was recommended for a BSGI examination for pre-surgical planning. A BSGI examination was also recommended for patients with negative or indeterminate mammographic findings.

The diagnostic results were compared with histological examination and the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each modality were determined. Combinations of either two or three of the diagnostic results were also reviewed and the corresponding diagnostic indicators calculated. For these

Table 1

	Sensitivity	Specificity	PPV	NPV
US	61.3	89.2	89.7	59.8
MMG	67.5	94.3	94.7	65.2
BSGI	83.1	87.9	91.4	77.1
US & MMG	86.8	84.7	89.8	80.6
US & BSGI	91.3	82.8	89.1	86.1
MMG & BSGI	92.2	83.4	89.5	85.6
US & MMG & BSGI	97.5	79.0	87.8	95.4

combinations, a positive indication on one of the two (or three) diagnostic tests were interpreted as a positive result. The sensitivity of US, MMG, BSGI, the combination of US and MMG, US and BSGI, MMG and BSGI, and the combination of all three together in the diagnosis of breast carcinoma were determined for the 400 patients included in the study and are listed in Table 1, column 1. The specificity, PPV, and NPV for each of these modalities and combinations of modalities were also determined and are shown Table 1, column 2-4.

The population was comprised of 243 patients with 245 malignant lesions and 157 patients with 189 benign lesions. The malignant lesions were primarily infiltrating ductal carcinoma (76%) and ductal carcinoma in situ (14%), with the remaining lesions (10%) being invasive lobular carcinoma, Paget's disease and mixed carcinomas. The benign lesions were primarily fibroadenoma (39%), adenosis (40%) and papilloma (10%), with the remaining lesions (11%) being duct ectasia, inflammation or phyllodes. There were 79 false negative cases by MMG, however, when ultrasound and BSGI were combined, 73 out of 79 were accurately identified. Additionally, there were nine, seventeen and eighteen Ductal Carcinoma in situ (DCIS) misdiagnosed by BSGI, MMG, and ultrasound respectively, but through joint imaging of BSGI+MMG+US; 33 out of 35 were accurately judged.

BSGI is a useful adjunct modality for the diagnosis of breast carcinoma. It is also extremely useful for diagnosis of non-calcified DCIS, and circumvents limitations of mammography in identifying malignant lesion in dense breast. Moreover, it is helpful for improving the diagnosis accuracy of breast carcinoma when BSGI is combined together with MMG and US.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-01-03

Title: Predictive value of FDG-PET/CT after neoadjuvant endocrine treatment in breast cancer

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Body: Background: Neoadjuvant endocrine therapy (NET) has demonstrated efficacy in terms of clinical response and outcome in hormone-receptor positive (HR+) post-menopausal patients (pts) with breast cancer (BC) not eligible for primary breast conservative surgery (BCS). However, the monitoring of tumor response to NET is challenging and clinical response is the current gold standard. The aim of the present study was to investigate the contribution of the early metabolic response (eMR) at one month in FDG-PET/CT in a NET setting for post-menopausal pts with HR+, HER2- BC compared to morphological and pathological responses. We also aimed to evaluate the prognostic value of eMR.

Methods: This was a prospective and ancillary study of CARMINA 02, UCBG0609 (*Cancer in press*), a phase II clinical trial evaluating the efficacy of 4 to 6 months neoadjuvant anastrozole or fulvestrant. FDG-PET/CT exams were performed at baseline (M0), after 1 month of treatment (M1: eMR) and pre-Op (late metabolic response: IMR) in 11 pts (74.2 years \pm 3.6) from 2007 to 2010. Pts were classified "metabolic responders" (mR) if SUVmax values decrease was \geq 40% at M1 and "non-metabolic responders" (mNR) if otherwise; IMR was also assessed in mR and mNR groups defined at M1. We compared eMR to morphological response (clinical, breast US and MRI) at M1 and pre-op, to the pathological response according to Sataloff classification and to Ki67 score variation during treatment. Early metabolic response was also correlated with the PEPI (Preoperative Endocrine Prognostic Index) score and survival (overall survival, OS and relapse free survival, RFS).

Results: Main results are summarized in Table I. There was a significant difference between mR and mNR pts at M1 (eMR) and pre-op (IMR). One patient with a complete metabolic response at pre-op had the best pathological response (Sataloff TB). Also, mR pts had a better clinical response: 2 partial response (PR) in mR vs 1 in mNR group and 2 mNR patients were classified PD (progressive disease). There was a trend toward better survival for mR pts in OS and RFS (Kaplan-Meier $p=0.18$ and 0.06 , respectively) and all the pejorative events occurred in the mNR group: 3 deaths and 3 metastatic progressions. Besides, no difference in eMR was observed regarding the histological subtype (ductal or lobular; $p>0.05$) nor the treatment group ($p>0.05$).

Table I: Metabolic, morphological and pathological response at M1, Pre-Op and on the surgical specimen.

		MR : 5pts	mNR : 6pts	P value
M1	SUVmax	2.6 \pm 1.1	3.9 \pm 1.4	0.00017
	Clinical size	42.5mm \pm 11.9	51.7mm \pm 7.5	0.19
	US size	22.6mm \pm 6.3	34.2mm \pm 2.4	0.02
	MRI size	21.2mm \pm 4.2	39.7mm \pm 4.7	9.16 E-5
	Ki 67	3.6% \pm 1.9	8.2% \pm 8	0.19
Pre-Op	SUVmax	2 \pm 1.3	3.3 \pm 1.4	0.018
	Clinical size	31mm \pm 12.4	48.3mm \pm 10.8	0.035
	US size	18.5mm \pm 7.3	31.3mm \pm 9.5	0.07
	MRI size	17.9mm \pm 7.1	34.8mm \pm 7.7	0.003
Surgical Specimen	Sataloff (TA+TB vs TC+TD)	20% vs 80%	0 vs 100%	1
	PEPI score (I+II vs III)	80% vs 20%	33 vs 67%	0.048
	Ki 67	8.6% \pm 9.8	12.3% \pm 7.9	0.41

Conclusions: These preliminary results showed the value of the early metabolic response in FDG- PET/CT in a NET setting

compared to the morphological or the pathological responses alone. Early metabolic responders patients had better OS, RFS and PEPI scores.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-01-04

Title: Assessing response in locally advanced breast cancer treated with neoadjuvant chemotherapy: Predictive and prognostic potential of volume-based metabolic variables with 18F-FDG PET/CT

Muñoz-Sánchez MdM, Soriano MdC, Garcia-Vicente AM, Molina-Garrido MJ, Pérez-Beteta J, Olaverri-Herández A, Molina-García D, Santiago Crespo JA, Pérez García VM, Val-Pérez E, Martín-Ordóñez F, Chacón-Muñiz JI, Álvarez-Cabellos R, Espinosa-Aunión R, Ortega-Ruipérez C, Cantero MJ, Castro A, Vaquero E, Catalá E, Torrecillas F and Soriano-Castrejón A. Hospital Virgen de la Luz, Cuenca, Spain; Hospital General Universitario de Ciudad Real, Ciudad Real, Spain; Instituto de Matemática Aplicada a la Ciencia. Universidad de Castilla-La Mancha, Ciudad Real, Spain; Servicio de Oncología Médica. Hospital Virgen de la Salud, Toledo, Spain and Servicio de Oncología Médica. Hospital La Mancha Centro, Alcázar de San Juan, Ciudad Real, Spain.

Body: Aim:

To explore the usefulness of metabolic variables of breast tumours obtained in 18F-FDG PET/CT in the prediction of response to neoadjuvant chemotherapy (NC) and prognosis in locally advanced breast cancer (LABC).

Material and methods:

Prospective multicenter study including 60 patients with LABC and NC indication and a baseline 18F-FDG PET/CT.

After breast tumor segmentation, SUV variables (SUVmax, SUVmean and SUVpeak) and volume-based variables as metabolic tumor volume (MTV) and total lesion glycolysis (TLG) were obtained.

Univariate analysis was performed with the use of Kaplan-Meier and Cox proportional hazards models to study the potential of metabolic variables, molecular phenotypes of breast cancer specimens and histologic response to predict: disease-free status (DFs), disease-free survival (DFS) and overall survival (OS).

Results:

Sixty patients underwent NC. 14 were classified as pCR. 56 patients were free of recurrence during the follow-up. Median \pm SD of DFS and OS was 43 \pm 15 and 46 \pm 13 months respectively.

SUV and volume-based variables showed significant relations ($p < 0.005$) with the histological response with higher values in responders compared to no responders (median, mean and SD of SUVmax, SUVmean, SUVpeak, MTV and TLG of 13.77, 13.34 and 5.79; 8.85, 8.35 and 3.73; 11.33, 10.7 and 4.88; 13.44, 26.60 and 34.58 and 96.39, 258.12 and 384.63 for responders vs 6.04, 7.84 and 5.26; 3.42, 4.77 and 3.20; 4.79, 6.06 and 4.08; 9.88, 13.63 and 12.48 and 33.78, 70.17 and 97.94 for non responders respectively).

MTV and TLG showed significant association with DFs ($p = 0.015$ and $p = 0.038$ respectively). Median, mean and SD of MTV and TLG for patients with DFs were: 8.90, 13.72 and 15.12 and 33.78, 90.54 and 144.64 respectively. Median, mean and SD of MTV and TLG for patients with a non-DFs were: 16.72, 29.70 and 31.09 and 90.89, 210.98 and 382.80 respectively. No significant relations were observed with SUV variables and DFs.

No significant relations were obtained for none metabolic variable with DFS and OS.

Conclusion:

In patients with locally advanced breast cancer, volume-based metabolic variables obtained with 18F-FDG PET/CT, differently to SUV based variables, were good predictors of neoadjuvant chemotherapy response and disease free status.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-01-05

Title: In vivo, intraoperative margin detection utilizing the Lumicell margin assessment system

Rai U, Tang R, Plichta JK K, Rice-Stitt T, Gadd MA A, Specht MC C, Strasfeld DS S, Ferrer JM M, Brachtel EF F and Smith BL L. Massachusetts General Hospital, Boston, MA and Lumicell, Inc., Wellesley, MA.

Body: ***This is a place-holder abstract***

In breast conserving surgery (BCS), obtaining tumor-free margins while excising minimal healthy tissue is critical for local control and maximizing cosmetic outcome. Currently, 20-40% of lumpectomy patients have positive margins that require surgical re-excision. Second surgeries increase discomfort, add emotional and cosmetic expenses to the patient, and add cost to the healthcare system.

This placeholder abstract will report the findings from Phase 2B of a two part Feasibility Clinical Study for the Lumicell Margin Assessment System, consisting of the LUM015 optical contrast agent and the LUM2.6 Imaging Device, for real-time, intraoperative detection of residual tumor in patients with breast cancer during lumpectomy surgery.

Phase 2A results demonstrated that LUM015 is tumor selective, safe in humans, and 100% sensitivity for tumor detection. Of 15 study patients, 10 were injected with LUM015, and 2 (20%) had positive margins with "ink on tumor" on standard histopathology and underwent re-excision. In both cases, the LUM Imaging System correctly identified residual tumor in lumpectomy cavity walls during the initial surgery which was confirmed by pathology. Data from Phase 2A were analyzed to determine the parameters of the detection algorithm which will be assessed in Phase 2B using a LUM015 dose of 1.0mg/kg.

In Phase 2B, up to 50 patients will be evaluated to achieve 10 evaluable subjects with positive margins based on histological margin assessment from the initial shaved margin. The surgeon will use the LUM 2.6 Imaging Device to remove up to three shaved margin specimens from each tumor bed cavity surface identified as containing fluorescence above the abnormal tissue threshold. Final margin assessment for each lumpectomy surface will be assessed per standard of care by a pathologist by measuring the distance to the inked surface of each tissue shaving from invasive cancer or ductal carcinoma in situ (DCIS). Concordance between final pathology determination and the LUM 2.6 Imaging Device will be reported along with overall positive margin rate. As of June 13, 2016, 9 patients have been enrolled, 3 patients have already undergone standard of care surgery followed by removing therapeutic shaves when indicated by the Lumicell Margin Assessment System.

We anticipate having the results from the Phase 2B study completed by the submission deadline of October 1, 2016. We will report the results in terms of concordance between intraoperative imaging and pathology analysis.

This study is registered with ClinicalTrials.gov, identifier NCT02438358.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-01-06

Title: Diffuse optical tomography can predict pathologic complete response in patients with HER2+ or triple negative breast cancer undergoing neoadjuvant chemotherapy

Kalinsky K, Lee S, Zhong X, Lim EA A, Gunther JE E, Hibshoosh H, Kim HK K, Accordino M, Crew K, Hielscher A and Hershman DL L. Columbia University Medical Center.

Body: Background

Pathologic complete response (pCR) predicts clinical outcome in women who receive neoadjuvant chemotherapy (NACT) for breast cancer. Identifying who will have a pCR early during NACT has the potential to save patients months of ineffective chemotherapy and limit unnecessary toxicity; however, no method currently is standardly used. Diffuse optical tomography (DOT) uses near-infrared light to measure concentrations of oxyhemoglobin [HbO_2], deoxyhemoglobin [Hb], total hemoglobin [HbT], and oxygen saturation [$\text{SO}_2\%$], and can assess tissue structure and vascularity. As it is inexpensive, fast, and does not require radiation or intravenous contrast no radiation nor IV contrast, DOT has the potential to become an integral part of NACT to predict responses to NACT. Given the particular significance for pCR in HER2+ and triple negative breast cancer (TNBC), we prospectively evaluated whether a 2 week change in DOT parameters could predict pCR after 5 months of NACT in these subtypes.

Methods

We conducted a prospective cohort study of women with stage II-IIIC breast cancer scheduled to receive NACT with 12 weeks of weekly taxol and four cycles of doxorubicin with cyclophosphamide (AC). We evaluated the associations between residual cancer burden (RCB: 0-3; pCR= RCB 0) and changes in DOT measures. Optical imaging was performed at baseline and before the following: Taxol #3, Taxol #5, AC #1, AC #2, and surgery. Correlation and t-testing were used to evaluate the relationship between 2-week DOT changes and pathologic response.

Results

In a prospectively accrued, longitudinal clinical study with DOT, at least 20 patients with HER2+ or TNBC were enrolled. For patients with these tumor subtypes, there was a significant association between pCR after 5 months of NACT (i.e. RCB 0) and change in the following DOT parameters comparing baseline to after 2 weeks of taxol: HBO ($p=0.02$), HBT ($p=0.02$), and $\text{SO}_2\%$ ($p=0.03$). No significant association was seen with HB ($p=0.20$) or water ($p=0.85$). When looking specifically at patients with TNBC (n =at least 8 patients), these associations were particularly strong between pCR and the following DOT parameters: HBO ($p=0.004$), HBT ($p=0.009$), and $\text{SO}_2\%$ ($p=0.04$). Additional patients are anticipated in this study are anticipated to complete NACT and will be reported at SABCS.

Conclusions

Optical imaging can provide imaging biomarkers to monitor breast cancer response to NACT. Early predictions of pathologic response to NACT can be made with high accuracy as early as two weeks after treatment initiation. These findings are specifically strong in TNBC, a group for whom pCR is predictive of clinical outcome.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-01-07

Title: A liquid biopsy test for breast cancer detection provides consistent diagnostic results in patients over six months

Benson KL L, Henderson MC C, Silver M, Gordon K, Borman S, Letsios E, Tran Q, Mulpuri R and Reese DE E. Provista Diagnostics, New York, NY.

Body: Current methods of breast cancer detection are often confounded by imaging limitations, such as lesion size, benign breast tissue, and dense breasts. These limitations result in unnecessary biopsies due to false positive findings based on imaging. Despite the increased ability to detect early breast cancer, the over-use of biopsy remains an issue. There is a critical need for new approaches to breast cancer detection that improve diagnostic accuracy when clinical assessment is challenging. Provista Diagnostics has developed Videssa® Breast - a blood-based proteomic test that measures serum protein biomarkers (SPBs) and tumor-associated autoantibodies (TAABs). Patient biochemical data is combined with clinical data to generate a diagnostic score that correlates with either the absence or presence of breast cancer (Grades I through III). The ability of Videssa® breast to detect cancer (Invasive Breast Cancer and Ductal Carcinoma *in situ*) was evaluated using prospective, multi-center clinical trials. The Provista-001 study enrolled 351 women ages 25-49 and included a follow-up visit at 6 months with an additional blood draw. Eligible patients included women assessed as ACR BIRADS® 3 or 4 on imaging with no history of breast cancer or prior breast biopsy.

Serum samples from the initial visit and 6 month follow-up visit of Provista-001 were analyzed using Videssa® Breast to determine if diagnostic results for benign subjects were similar over the course of the study. Samples were analyzed for SPBs and TAABs in order to determine whether analyte levels and diagnostic scores change over a 6-month period in patients diagnosed with a benign breast condition. Linear regression data for analytes shows overall high fidelity between the initial visit and follow-up. In addition, samples that were TAAB-positive for a given target at the initial visit tended to remain positive at follow-up. Sample background, deriving from unidentified immunological factors, can confound the analytical output when measuring TAABs in serum. Interestingly, sample background was highly reproducible between both visits, suggesting that these values are related to inherent patient-specific factors. Overall, these data demonstrate high analytical reproducibility for in expression of independent Videssa® Breast biomarkers in patients diagnosed with a benign breast condition over the course of six months. Data for 236 women were compared between visits and demonstrated greater than 80% concordance in diagnostic status.

The ability of Videssa® breast to provide consistent diagnostic results over 6 months further supports use of the test as an adjunct to imaging for the early detection of breast cancer and provides physicians with an additional tool that can be used to inform the decision to biopsy or increase vigilance through active monitoring.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-01-08

Title: Specific detection of anti-Her2 PEGylated PrecisionMRX® nanoparticles measured using superparamagnetic relaxometry

Weldon CL L, Minser KE E, Gomez A, Anderson WH H, Karaulanov T, Hathaway HJ J, Huber DL L, Vreeland EC C and Paciotti G. Senior Scientific LLC, Albuquerque, NM; University of New Mexico Health Sciences Center and University of New Mexico Comprehensive Cancer Center, Albuquerque, NM and Center for Integrated Nanotechnologies, Sandia National Laboratories, Albuquerque, NM.

Body: Current methods for detecting solid tumors lack sensitivity and diagnose primary and metastatic lesions only after the tumor is well established. Superparamagnetic Relaxometry (SPMR) is a combination technology that utilizes superconducting quantum interference detectors (SQUID) to measure the magnetization of superparamagnetic, tumor-targeting magnetite (Fe_3O_4) nanoparticles. Conceptually, PEGylated Fe_3O_4 nanoparticles labeled with a tumor targeting moiety (i.e., a monoclonal antibody) are intravenously injected and specifically target solid tumors utilizing both passive (the EPR effect) and active (receptor-mediated) mechanisms. Subsequently, the Fe_3O_4 nanoparticles are magnetized by a low field magnetic pulse in the MRX™ instrument and only those particles that are bound to their target site are measured by the SQUID sensors. Unbound nanoparticles are not detected.

To demonstrate the utility of SPMR in detecting cancer we used PEGylated PrecisionMRX® nanoparticles that are covalently linked with a monoclonal antibody (mAb) targeting ERB-2 (anti-Her2). The particles were characterized for size (by dynamic light scattering), free and bound mAb (by ELISA), antibody potency (by bioassay) and stealth (in plasma interaction studies). In vitro, the anti-Her2 conjugated particles exhibited specific binding to ERB-2 overexpressing breast cancer cells (MCF-7/Her2-18). Specific binding was defined by the ability of the native mAb to competitively block the binding of the anti-HER-2 conjugated particles to ERB-2 antigen coated on ELISA plates or expressed on the cell surface. In addition, in ERB-2 negative cell lines, the anti-Her2 conjugated particles exhibited little to no binding.

In vivo, anti-Her2 conjugated PrecisionMRX exhibited significantly longer circulation times when compared to unPEGylated particles. Distinct magnetic dipoles were detected by the MRX instrument at the target site (the tumor) and site of nanoparticle elimination (the liver). These data were confirmed in excised organs showing significant magnetic moments in the liver, tumor, and spleen.

Analysis of the MRX SPMR data suggest that the technology can detect as few as 10,000 cancer cells in vivo by optimizing the nanoparticles for stealth and targeting.

This work was performed, in part, at the Center for Integrated Nanotechnologies, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science. Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-01-09

Title: γ -glutamyl hydroxymethyl rhodamine green (gGlu-HMRG) is promising fluorescence probe for rapid diagnosis of breast cancer; - The feasibility study of real time imaging for breast cancer examination -

Takamaru T, Akashi ST T, Kuwayama T, Sawada T, Hirota Y, Urano Y and Nakamura S. Showa University Koto Toyosu Hospital, Tokyo, Japan; Showa University, Tokyo, Japan and Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

Body: Background and Aim

To date, fluorescence imaging has been used gradually for real time diagnosis in various clinical situations. Evaluation of margin on surgical specimens is essential to decide whether additional resection should be performed for breast cancer surgery. In the same context, rapid assessment of biopsy specimen is crucial because when they do not contain any part of the lesions, re-examination should be need.

A fluorescence probe named γ -glutamyl hydroxymethyl rhodamine green (gGlu-HMRG) was rapidly activated by an enzyme, γ -glutamyltransferase (GGT). It was overexpressed in variety of cancers so promising for clinical use. The aim of this study is to examine the usefulness of the probe for breast cancer detection.

Material and Methods

We investigated the patients of breast cancer or benign disease who received examination consecutively from March 2015 to February 2016 in our hospital. The samples were obtained by core needle biopsy (CNB) or vacuum-assisted breast biopsy (VAB). We sprayed the probe on these samples immediately after examination and shoot images by CCD camera. To evaluate the fluorescence intensity along the time, we used a filtered CCD camera that could detect specifically gGlu-HMRG color (Discovery® INDEC Medical Systems Inc.). The images were automatically obtained every 30 seconds for 10 minutes after adding the probe on the specimens.

The average value of the image in each region of interest (ROI) was analyzed using image analysis program Image J (<https://imagej.nih.gov/ij/>).

We investigated the change of fluorescence intensity with the passage of time. We also compared the fluorescence intensity of malignant lesions with benign ones, and analyzed whether the fluorescence intensity could distinguish the malignant lesions from benign ones.

Result

We obtained 362 samples from 96 tumors. Fifty-six tumors with 215 samples were benign, while 40 tumors with 147 samples are malignant histologically.

The fluorescence was immediately observed after sprayed the probe. The intensity had been increasing in proportion to time. The malignant specimens were rapidly increasing; in contrast, the benign ones were slowly. For example, when it took 60 seconds after spraying the probe that the intensity increase up to some level in malignant specimens, while benign one took 240 seconds up to the same level on average.

Comparing the malignant lesions with benign ones after sprayed 120 seconds, the fluorescence intensity was higher in malignant specimens than benign ones (average fluorescence intensity; benign 0.9, malignant 2.3 $p=0.0138$). By ROC analysis whether the fluorescence intensity could distinguish the malignant lesions from benign, AUC, sensitivity and specificity was 0.63, 70% and 57%, respectively (cut off 0.2).

Conclusion

The probe was contributory to distinguish malignant and benign lesions and may be useful for the rapid diagnosis of CNB in practice. We are now trying to seek a more accurate probe to differentiate benign and malignant lesion as a next step.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-01-10

Title: Development of photoacoustic vascular imaging system for breast cancer

Toi M, Asao Y, Takada M, Kataoka M, Endo T, Kawashima M, Yamaga I, Nakayama Y, Tokiwa M, Fakhrejahani E, Torii M, Kawaguchi-Sakita N, Kanao S, Matsumoto Y, Yagi T, Sakurai T, Togashi K and Shiina T. Graduate School of Medicine, Kyoto University, Kyoto, Japan; Medical Imaging System Development Center, Canon Inc., Tokyo, Japan; Graduate School of Medicine, Kyoto University, Kyoto, Japan; Graduate School of Medicine, Kyoto University, Kyoto, Japan and Graduate School of Medicine, Kyoto University, Kyoto, Japan.

Body: Background:

Tumor angiogenesis and hypoxia are associated with breast cancer growth and metastasis. Photoacoustic (PA) tomography is an optical imaging technology that visualizes distribution and oxygenation status of hemoglobin with high spatial resolution. Initially we developed a photoacoustic mammography (PAM) having a flat-shaped scanning detector that could detect breast tumors. Nevertheless, the flat-shaped detector array has the drawback of a limited view. Here we developed a novel PAM system with a hemispherical-shaped detector array (HDA), which enables us to identify microvasculatures non-invasively and allow the collection of nearly spatially isotropic three-dimensional reconstructed image of blood vessels. This non-invasive vascular imaging system may be able to characterize tumor angiogenesis and analyze the status of microcirculation. The aim of this study was to analyze the imaging findings of tumor-related vasculature in breast cancer patients.

Patients and method:

A PAM system with HDA has been generated in a cooperation project between Canon Inc., Japan, and Kyoto University. Twenty-two primary breast cancer patients, including 5 patients with non-invasive cancer and 17 patients with invasive cancer, diagnosed between December 2014 and December 2015 underwent the PAM imaging analysis. We also applied the breast deformation algorithm from the breast shape in a MRI image to that in a PA image in order to create a fusion image of the two modalities for the analysis. Features of peri- and intra-tumoral vasculature, and their oxygenation status were evaluated. The study protocol was approved by the institutional review board at Kyoto University Hospital (UMIN000012251). All patients provided informed consent to participate in this study.

Results:

The abnormal peri-tumoral vasculature was detected in 86% of all non-invasive and invasive disease cases. In invasive cancer cases, most tumor-related blood vessels were centripetally directed toward the tumor, and 93% of centripetal blood vessels appeared to be disrupted or rapidly narrowed at the tumor boundary. The centripetal blood vessel structure was frequently observed in invasive cancer compared with non-invasive cancer (61% vs 35%). PA images before and after preoperative chemotherapy were obtained in one case, where intra-tumoral blood vessels became finer after chemotherapy, reflecting normalization of intra-tumoral microcirculation induced by chemotherapy.

Conclusions:

A PAM system with HDA has provided a high-resolution vascular images of primary breast cancers. The morphological differences of peri-tumoral vasculature were observed between invasive disease and non-invasive disease. These results suggest the potential of PA imaging as a non-invasive tool to analyze tumor vasculature of human breast cancers and maybe be helpful for breast cancer diagnosis.

(Acknowledgements)

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2016 San Antonio Breast Cancer Symposium

Publication Number: P4-01-11

Title: Diagnostic performance of supplemental screening with molecular breast imaging in women with dense breast tissue

Redfern RE E, Shermis RB B, Chen JT T and Kudrolli H. ProMedica Toledo Hospital, Toledo, OH; Bowling Green State University, Bowling Green, OH and Gamma Medica, Inc, Salem, NH.

Body: Purpose: The aim of this study was to retrospectively assess the diagnostic performance of molecular breast imaging (MBI) as a supplementary screening tool for women with dense breast tissue. We had previously reported excellent cancer detection rates and positive predictive values, whereas in this report we present 1-year follow up data and present sensitivity, specificity and negative predictive values. Methods: Molecular Breast Imaging (MBI) was implemented in routine clinical practice at a large, community-based breast imaging center. Women 25 to 90 years of age with dense breasts and negative mammogram results who underwent subsequent screening with 300 MBq (8 mCi) ^{99m}Tc -sestamibi MBI were retrospectively analyzed. Outcome measures included sensitivity, specificity, and positive and negative predictive values. Results: In 1,376 women with a complete reference standard, 17 (1.2%) were diagnosed with cancer. MBI detected cancer in 15 subjects such that 2 interval cancers occurred and were considered false negatives. Of those 17 cancers, 1 (5.9%) were ductal carcinoma in situ only, 13 were invasive tumors (76.5%); 1 (5.9%) patient was diagnosed with bilateral disease. Overall sensitivity of MBI in this sample was 88.2% (95% CI 63.6 – 98.5), while specificity was also high at 92.4% (95% CI 90.9 – 93.8). Positive predictive value for recall (PPV_1) was 12.7% (95% CI 7.3 – 20.1), while negative predictive value for recall (NPV_1) was calculated as 99.8% (95% CI 99.4 – 100.0). Conclusions: When incorporated into a community-based clinical practice environment, MBI demonstrated high sensitivity, specificity and negative predictive value. These results demonstrate the utility of MBI as a supplementary screening tool to mammography for women with dense breasts.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-01

Title: Eribulin induces vascular remodeling and reoxygenation in advanced breast cancer patients: A comparative study with bevacizumab

Ueda S, Saeki TS, Takeuchi H, Shigekawa T, Yamane T, Kuji I and Osaki A. International Medical Center, Saitama Medical University, 1371-1 Yamane, Hidaka, Saitama, Japan.

Body: Purpose: Eribulin mesylate (eribulin) is a first-in-class halichondrin B-based microtubule dynamics inhibitor. To understand the mechanism of vascular remodeling of eribulin, we compared optical hemodynamic and blood biomarker changes between eribulin and bevacizumab.

Methods: Patients with advanced breast cancer with stage III/IV were eligible for the study. Patients were assigned to receive either eribulin or single-agent bevacizumab. Diffuse optical spectroscopic imaging (DOSI) measured tissue concentrations of oxy-hemoglobin (O₂Hb), deoxy-hemoglobin (HHb), total hemoglobin (tHb) and oxygen saturation (SO₂) of breast tumors before and day 7 after the first infusion. Peripheral blood samples were obtained from the patients to measure plasma concentration of VEGF, bFGF, FLT-3L, EGF, G-CSF, TNF α , IL1b, IL4, IL6, IL8, IL10, IL12p40, and TGF- β 1.

Results: Baseline DOSI measurement of all 29 patients (eribulin, n = 14 and bevacizumab, n = 15) revealed significantly higher tumor concentrations of O₂Hb, HHb, and tHb than that in the normal breast tissue. Eribulin significantly decreased in HHb concentration and increased SO₂ during the observation period. This trend was not observed for bevacizumab. Instead, bevacizumab significantly decreased the concentration of O₂Hb and tHb. The blood biomarker study revealed that both eribulin and bevacizumab decreased plasma concentrations of VEGF and bFGF, but only eribulin suppressed the plasma concentration of TGF- β 1.

Conclusions:

Optical imaging technology revealed that eribulin, but not bevacizumab, induced tumor reoxygenation after the start of infusion. Eribulin had a potent anti-angiogenic properties as well as bevacizumab, while suppression of TGF- β 1 observed by only eribulin could be associated with remodeling of the microvasculature through suppression of activated stromal cells.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-02

Title: Increased tumor perfusion following treatment with trastuzumab as measured by contrast-enhanced ultrasound

Sorace AG G, Barnes SL L, Quarles CC Chad, McIntyre JO Oliver and Yankeelov TE E. The University of Texas at Austin, Austin, TX; St. Joseph's Hospital and Medical Center, Barrow Neurological Institute, Phoenix, AZ and Vanderbilt University Medical Center, Nashville, TN.

Body: Introduction: The primary purpose of this study is to determine if the order of dosing in standard-of-care (SOC) combination therapy for HER2+ breast cancer has an effect on the perfusion characteristics within the tumor. Improving the intratumoral delivery of cytotoxic systemic therapy is a significant challenge in advancing cancer treatment. Knowledge of the vascular changes following SOC treatments could enable optimizing their order and timing, potentially leading to significantly improved response. Currently, one SOC regimen for HER2+ breast cancer treatment is doxorubicin administered for 3-4 cycles prior to trastuzumab. Our goal is to quantitatively map the changes in perfusion in response to different combinations of trastuzumab plus doxorubicin treatment through imaging in a murine model of HER2+ breast cancer.

Experimental Design: BT474 breast cancer cells (1×10^7) were subcutaneously implanted into mice ($n = 12$) and randomly assigned into three treatment groups: two doses of trastuzumab (10 mg/kg) followed by doxorubicin (1.5 mg/kg), doxorubicin prior to trastuzumab (same total drug dosage as group 1), and saline. After tumors reached $\sim 225 \text{ mm}^3$, animals were imaged with contrast-enhanced ultrasound (CEUS) (VisualSonics Vevo 770, Definity microbubbles) before treatment (day 0), and on days 1, 3, 4, and 7. Treatment occurred on days 0, 3 and 4. Percent change (from baseline, day 0 scans) of the CEUS signal intensity quantified from the functional vasculature (surrogate for vessel perfusion) following contrast injection were measured for each animal for each day. Tumors were extracted on day 7, and sectioned, paraffin-embedded, and stained with CD31, alpha-SMA and H&E.

Results: Tumors treated with trastuzumab initially exhibited a significant increase in CEUS signal intensity (from the functional vasculature) on day 1 compared to tumors initially treated with doxorubicin ($p < 0.01$). Additionally, compared to the control tumors, tumors treated with trastuzumab prior to doxorubicin revealed a significant increase in perfusion (change in signal intensity of functional vasculature) of contrast agent on days 3 ($p = 0.01$), 4 ($p = 0.001$) and day 7 ($p < 0.01$). There were no significant differences in the doxorubicin treated first group and the controls on any of the days ($p > 0.25$). Qualitative differences were noted between control and treated groups for alpha-SMA, no apparent differences were noted in microvessel density.

Conclusion: Trastuzumab significantly improves a tumor's vascular perfusion in this HER2+ breast cancer model. Doxorubicin dosing prior to treatment with trastuzumab may potentially be hindering the intratumoral delivery of the subsequently delivered, targeted therapy. Improving the tumor's functional vasculature by altering the order of dosing of these combination therapies by giving trastuzumab prior to cytotoxic therapy has potential to enhance both the delivery and the effectiveness of these combination therapies. These data indicate a potential pathway to optimize therapeutic efficacy for individual HER2+ breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-03

Title: Breast biopsy histology relationships with opto-acoustic imaging of breast masses

van de Vijver M, Lavin PT T and Stavros AT T. AMC, Amsterdam, Netherlands; Boston Biostatistics Research Foundation, Framingham, MA and Seno Medical Instruments, Inc., San Antonio, TX.

Body: Purpose: The Imagio® breast imaging system, a diagnostic opto-acoustic (OA) imaging device bearing the CE Mark, is in the U.S. FDA Premarket Approval process. OA provides both functional (relative oxygenation/de-oxygenation) and anatomic (angiogenesis) information that is co-registered and temporally interleaved in real time with gray-scale ultrasound that may improve distinction between benign and malignant masses. OA imaging pathology correlation was performed to elucidate the histologic features of OA features of breast cancers.

Methods and Materials: A multicenter postmarket surveillance and clinical follow-up study was conducted in five Dutch sites in which 209 women with breast masses underwent OA prior to biopsy. Histopathology examination of the biopsies revealed 146 benign masses (mostly fibroadenomas) and 76 malignant masses (mostly invasive ductal carcinomas). For invasive ductal carcinomas, histologic grade and the features used to assess histologic grade (nuclear pleomorphism, tubule formation, and mitotic count) were assessed. For each mass, 5 pre-determined OA features, 3 internal features, and 2 external features were evaluated. The 3 internal scores (vessels, blush, and hemoglobin) and 2 external features (capsular boundary zone and peripheral boundary zone) were separately and collectively summed for testing relationships with traditional histopathology measures using a two-sided Jonckheere-Terpstra test of ordered outcomes. Distribution differences between benign and malignant masses were performed using a Wilcoxon Rank Sum test for each internal, external, and summed total internal, external, and total score.

Results: The mean differences were significantly higher for malignant vs. benign for internal vessels ($p=0.0009$), internal blush ($p=0.0085$), external boundary zone ($p<0.0001$), and external peripheral zone ($p<0.0001$), but not internal hemoglobin. Mean Total Internal Score, Total External Score, and Total Internal and External Score were all significantly higher (all $p<0.01$) for malignant vs. benign. Among invasive carcinomas, Total Internal Score and Total Internal and External Score were significantly higher for higher histologic tumor grade ($p=0.04, 0.02$), significantly higher Total External Score and Total Internal and External Score for higher tubule score ($p=0.06, 0.03$), slightly higher Total Internal Score and Total Internal and External Score for higher nuclear pleomorphism score ($p=0.04, 0.05$), and slightly higher Total Internal Score for higher mitotic score ($p=0.03$).

Conclusion: OA feature summary scores appear to differentiate between benign vs. malignant and correspond to histologic grade and scoring components of histologic grade. The U.S. investigational PIONEER pivotal study ($n=2,095$) may further confirm these results.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-04

Title: Using ultrasound findings to predict high tumor-infiltrating lymphocytes in triple negative breast cancer

Inagaki M, Ota D, Tsuji M, Kobayashi Y, Mori M and Fukuuchi A. Breast and Endocrine Surgery, Mitsui Memorial Hospital, Tokyo, Japan and Pathology, Mitsui Memorial Hospital, Tokyo, Japan.

Body: Purpose Previous clinical data showed that high tumor-infiltrating lymphocytes (TILs) indicated a good prognosis in triple negative breast cancer (TNBC) and suggested that TNBC with TILs responded well to immunotherapy. Breast ultrasound is a safe inspection method without any radiation exposure. Therefore, we investigated whether ultrasound findings can predict high TILs in TNBC.

Patients and Methods The TNBC patients who underwent surgery at Mitsui Memorial Hospital, Tokyo, Japan, from 2009 to 2015, including those who underwent neo adjuvant chemotherapy (NAC), were selected for this study. The pre-treatment ultrasound findings were used retrospectively, and we compared the shape, margin, homogeneity of internal echoes, posterior features, and growth pattern of the lesions. Regarding shape, we classified the ultrasound findings as "round", "oval", "lobulated", "irregular", and "other" in accordance with The Japan Associations of Breast and Thyroid Sonology. Similarly, we classified the margins as "circumscribed" or "well-defined and smooth", "well-defined and rough", "indistinct" and "obscure". We used "homogeneous" and "heterogeneous" internal echoes. In terms of posterior features, we used the "accentuation", "not changing", "attenuating" and "shadowing" categories. By referring to previous report, we classified four types of growth patterns, i.e., "growing along the mammary ducts", "expansive growth pattern", "irregularly shaped mass with retracting surrounding tissue" and "other". Two pathologists evaluated stromal TILs, which were located between the cancer nests, by surgical specimens or needle biopsies of those who underwent NAC. Then the TILs were classified into four groups: "absent", "weak", "moderate", and "dense". "Absent" indicated that there were no lymphocytes in the stroma. "Weak", "moderate" and "dense" indicated that lymphocytes occupied about 1–40%, 40–75% and 75%–100% area in the stroma, respectively. We analyzed ultrasound findings and TILs with the χ -square test.

Results A total of 97 lesions and 95 female TNBC patients were validated. The median age was 62 years old (range, 32–88 years). Of the total, 37 patients underwent NAC. The degree of "absent" TILs was 5 lesions, "weak" was 58, "moderate" was 22, and "dense" was 12 lesions. In the ultrasound findings, the shape categories "round," "oval," and "lobulated" were more "dense" TILs (n=12, 100%) than others (n=47, 55.3%) (p = 0.002). The "circumscribed" and "well-defined and rough" margins were found to be more "dense" TILs (n=11, 91.7%) than the other TILs (n=49, 57.6%) (p=0.020). "Accentuating" posterior echoes were more "dense" and "moderate" TILs (n=23, 67.6%) than "weak" and "absent" TILs (n=25, 39.7%) (p=0.009). The lesions with expansively growing pattern showed higher rate "dense" and "moderate" TILs (n=20, 58.8%) than "weak" and "absent" TILs (n=20, 31.7%) (p=0.010). There were no significant differences in internal echoes.

Conclusion We determined that ultrasound findings of round, oval or lobulated shape, accentuating posterior echoes and expansively growing pattern could predict the presence of high TILs. Thus, the safe, low-cost, and radiation-free ultrasound examination was recommended for predicting high TILs and prognosis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-05

Title: Test-retest fidelity of FDG SUVmax in bone and non-boney metastatic breast cancer lesions in local area network PET/CT scanners

Linden HM M, Peterson LM M, Kurland B, Roberts T, Specht J, Shields AT T, Novakova A, Christopfel R, Byrd D, Muzi M, Mankoff DA A and Kinahan P. Medical Oncology, University of Washington Medical Center, Seattle, WA; Biostatistics, University of Pittsburgh, Pittsburgh, PA; Radiology, University of Washington Medical Center, Seattle, WA and Radiology, University of Pennsylvania, Philadelphia, PA.

Body: Background: Metabolic activity in lesions, measured by FDG-PET, is often used for assessing tumor aggressiveness and response to therapy. Patients may be scanned on different machines, so quantitative measurements should be reproducible. Reducing SUV variability in PET machines throughout a local network can aid in monitoring patient response to therapy and increase access to clinical trials.

Methods: Eighteen female patients with advanced or metastatic breast cancer underwent paired FDG PET/CT test-retest studies with 1-15 days between scans, and without interim change in treatment. Ten patients were studied in the same scanner and 8 patients were studied in 2 different scanners. Five different PET/CT scanners were used (2 GE DSTE, 2 Siemens (BioGraph 6 and mCT), 1 Philips Ingenuity TF). Each PET/CT scanner was calibrated using NIST-traceable reference sources to characterize and reduce variability. All of the images were interpreted by two separate reviewers. SUVmax values in lesions, corresponding normal tissue, and normal liver were collected. Linear mixed models with random intercept (patient effects) were fitted to compare differences in $\log(|\text{SUVmax \% difference}| + .01)$ in multiple lesions per patient.

Results: SUVmax was assessed in a total of 130 lesions (75 bone). The median number of lesions per patient was 5 (range 1-17). Average SUVmax ranged from 1.0 to 18.2 ($\text{mean} \pm \text{SD} = 6.0 \pm 3.2$). The median SUVmax difference was 0.4 (8%) for 47 lesions imaged twice in the same scanner, and was 0.6 (13%) for 83 lesions imaged in two different scanners. In a multivariable linear mixed effects model, SUVmax for different scanners within the same institution did not differ more than for the same scanner ($p=0.39$), but repeat scans with different scanners and site personnel at had an average of 78% greater percentage difference in SUVmax than for the same scanner ($p=0.009$). In the same model, the average percent difference in SUVmax for bone lesions was estimated as 30% lower than for other sites ($p=0.06$, 95% confidence interval 0-50%). Examining normal liver uptake, the median SUVmean was 2.5 (range 1.9-3.1) with an median 6.5% difference between measurements (range 1.1%-23.7%) that did not appear to differ based on scanners used for repeat measurements ($p=0.47$).

Conclusions: The variability in quantitative FDG SUVmax between scans is modest, suggesting reliable reproducibility in appropriately calibrated settings. In our study, bone lesions had somewhat higher fidelity than other tumor sites. Additional studies will address variability in other cancer types. Careful calibration and monitoring of PET/CT scanners, and consistent imaging protocols are necessary in clinical trials that utilize quantitative PET/CT imaging in order to confidently interpret results.

Research Support: NIH grant U01-CA148131 and NCI-SAIC Contract 24XS036-004.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-06

Title: Evaluation of contrast-enhanced ultrasonography for early prediction of response to neoadjuvant chemotherapy in triple negative breast cancer

Masumoto N, Kadoya T, Murakami C, Gouda N, Sasada S, Emi A, Haruta R, Kataoka T and Okada M. Hiroshima University and Hiroshima University Hospital.

Body: Objectives

We aimed to determine whether contrast-enhanced ultrasonography (CEUS) can predict the early effects of neoadjuvant chemotherapy on triple negative breast cancer.

Methods

The clinical responses of 20 consecutive patients with breast cancer (T1–2, N0–1, M0) to neoadjuvant chemotherapy between October 2012 and Feb 2016 were assessed using ultrasonography and contrast-enhanced ultrasonography before starting the therapy and after the treatment of 2 courses. Ascending slope (AS) of perfusion parameters for contrast-enhanced ultrasonography were created from time–intensity curves based on enhancement intensity and temporal changes to objectively evaluate contrast-enhanced ultrasonography findings. We investigated whether rate of change of ascending slope (Δ AS) and tumor size (Δ US) could predict pCR.

Results

Eight (40.0%) of the 20 patients achieved pathological complete response. Δ AS were significantly higher (-25.5 ± 35.5 vs. 14.7 ± 33.2 ; $P < 0.02$) in patients who achieved pCR than in those who did not. On the other hand, Δ US of pCR and non-pCR did not significantly differ among tumors (-40.8 ± 22.4 vs. -21.4 ± 20.6 ; $P = 0.06$). The AUC values for Δ AS and Δ US were 0.792 (95% CI, 0.579 -1.000, $P = 0.03$) and 0.729 (95% CI, 0.501 - 0.957; $p = 0.09$), respectively. We set Δ AS and Δ US cut-offs for predicting pCR at -20.08 and - 33.75 based on the ROC curves. Clinical and pathological characteristics of the 20 patients are summarized in

Clinical and pathological characteristics of patients with breast cancer.

Characteristic	pCR (n)	Non pCR (n)	p
Clinical T status			
T1	1	1	0.71
T2	7	11	
Clinical N status			
Negative	5	7	0.85
Positive	3	5	
Nuclear Grade			
1 or 2	3	4	0.85
3	5	8	
Δ US (%)			
< -33.75	3	9	0.09
\geq -33.75	5	3	
Δ AS (%)			
< -20.08	2	10	0.009
\geq -20.08	6	2	

. Univariate (odds ratio, 2.71; $p = 0.02$) and multivariate (odds ratio, 2.88; $p = 0.03$) analysis showed that Δ AS was the sole

independent predictor of pCR

Univariate and multivariate logistic analysis of significant predictive factors for pCR in triple negative subtype

	Univariate analysis	Multivariate analysis
	OR, 95% CI, p	OR, 95% CI, p
Δ US		
> -33.75	1.61, 0.72-34.7, 0.10	1.85, 0.51-79.1, 0.15
\leq -33.75		
Δ AS		
> -20.08	2.71, 1.65-136.1, 0.02	2.88, 1.44-218.7, 0.03
\leq -20.08		

Conclusion

Δ AS assessed with CEUS can help the physician to early predict the probability of achieving pCR or not.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-07

Title: Preoperative assessment of breast cancer survival using ultrasound diameter and shear wave elastography

Evans AJ J, Purdie C, Thompson AM M, Jordan L, Fuller-Pace FV V, Whelehan PJ J, Macaskill JE E and Vinnicombe S. Dundee University, Dundee, Scotland, United Kingdom and MD Anderson Cancer Centre, Houston, TX.

Body: Introduction: assessment of prognosis has traditionally been performed after surgical excision of breast cancer. However with the increased use of neoadjuvant chemotherapy (NACT) there is a need for pre-operative prediction of prognosis to aid treatment selection. Stiffness at shear wave elastography (SWE), an ultrasound technique, has been shown to correlate with histologic grade, independently predict nodal involvement and be associated with response to NACT. SWE is easy and fast to perform, is quantitative and has been shown to be highly reproducible. We therefore hypothesized that lesional stiffness and size at pre-operative US examination may be associated with breast cancer mortality and provide useful pre-treatment prognostic information.

Methods: consecutive patients undergoing breast ultrasound (US) for lesions subsequently shown to be invasive breast cancer between April 2010 and December 2011 underwent SWE had the US lesion diameter and mean stiffness (kPa) at SWE recorded prospectively. Patient's survival including cause of death was ascertained from local and national sources. Eight patients with metastases at presentation were excluded. Breast cancer specific survival (BCSS) was ascertained for 3 equal groups based on US size, mean kPa, and a combination using equal weighting of the 2 parameters. BCSS was assessed using Kaplan-Meier survival curves and statistical significance ascertained using the logrank test.

Results: 289 patients (mean age 63 yrs, 107 screen detected and 182 symptomatic lesions) constituted the study group. 20 breast cancer and 21 non-breast cancer deaths were recorded and mean follow-up in those alive was 4.8 years. Breast cancer survival for equal size groups based on a) stiffness were 98%, 91% and 89% ($p=0.05$) b) US size were 98%, 95% and 85% ($p=0.001$) and c) a combination of US size and stiffness were 99%, 97% and 82% ($p<0.0001$).

Conclusion: the combination of US diameter and mean stiffness at SWE provides powerful preoperative assessment of prognosis in women with breast cancer. This information could be used to inform decisions regarding NACT.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-08

Title: Opto-acoustic imaging of the breast: Downclassification and upclassification of suspicious breast masses

Menezes GLG L G, Pijnappel RM M, Meeuwis C, Bisschops B, Veltman J, Lavin PT T and Mann RM M. University Medical Centre Utrecht, Utrecht, Netherlands; Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; Albert Schweitzer Hospital, Dordrecht, Netherlands; ZGT Almelo, Almelo, Netherlands and Lavin Consulting LLC, Framingham, MA.

Body: Background

Imagio® is an opto-acoustic (OA) breast imaging system designed to concomitantly collect OA images together with diagnostic ultrasound (CDU). The device is intended to improve distinction between benign and malignant masses. In this interim analysis of the post-market surveillance MAESTRO study we report the results of 75 patients who had breast masses classified as BI-RADS 4a and 4b by CDU.

Aim

We assessed OA's sensitivity, specificity, and its ability to downgrade benign masses and upgrade malignant masses based on the probability of malignancy (POM) and BI-RADS category.

Methods

Seventy-five patients with 78 breast masses were evaluated with OA prior to biopsy. For each mass, the radiologist scored 5 OA features, assigned a POM and BI-RADS category. OA sensitivity, specificity, and BI-RADS downgrade and upgrade percentages were assessed with and without contribution from a previously derived nomogram.

Results

The mean POM difference between malignant and benign masses was higher for OA (37%) than for CDU (27%). OA specificity was 43% without the nomogram and 68% with the nomogram. OA sensitivity was 97% with and without the nomogram. With OA 43% of benign masses could be downgraded and 47% of malignant masses could be upgraded in BIRADS category.

Conclusion

These results appear to confirm the previously reported ability of OA to improve the differentiation between benign and malignant masses compared to CDU alone, to potentially decrease negative biopsies, and to upgrade BI-RADS category in malignant masses. The MAESTRO study (n=200) may further confirm these results.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-09

Title: Non-invasive and low-cost technique for early detection of clinically relevant breast lesions using a handheld point-of-care medical device (iBreastExam): Prospective three-arm triple-blinded comparative study for breast cancer screening in low resource setting countries

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Body: **OBJECTIVE:** To determine the clinical efficacy of a handheld point-of-care medical device that could potentially assist allied healthcare workers to perform standardized Clinical Breast Examination in low-resource settings.

MATERIALS AND METHODS: Nine hundred and eighty-nine healthy women visiting a tertiary Indian hospital, for annual health check were recruited for bilateral breast examinations. Additionally, 20 women attending the hospital with breast-related symptoms were also recruited as part of the opportunistic screening program. Each woman was examined by three independent methods, each blinded to the other two: iBreastExam (iBE), Clinical Breast Examination (CBE) by an expert clinician and Breast Imaging (mammography or breast ultrasound). Main Outcome Measures Sensitivity, Specificity, PPV, NPV for iBE and CBE were derived with Breast Imaging tests used as reference standard.

RESULTS: Out of 916 enrolled participants, 93 were confirmed by imaging to have at least one breast lesion. Clinical Breast Examination in comparison with imaging detected breast lesions with Sn = 65 %, Sp = 94 %, PPV = 52 %, NPV = 96 %, and iBreastExam reported Sn = 84 %, Sp = 94 %, PPV = 60 % and NPV = 98 %. In women below age 40 (314 participants), iBE detected breast lesions with Sn = 85 %, Sp = 93 %. All malignant lesions were identified by iBE, while one non-palpable malignant lesion was missed by clinician CBE.

CONCLUSION: The point-of-care Breast Imaging device (iBreastExam) performed with significantly better sensitivity, by 19 %, than CBE to detect breast lesions while reporting high specificity (94 %) and NPV (98 %). In younger women population under the age of 40 years, where the prevalence of dense breast is high, iBreastExam demonstrated high-performance characteristics. iBreast- Exam detected all malignant lesions in this study, while the clinician's CBE missed to detect a non-palpable malignant lesion. iBreastExam can be a promising tool to provide clinically effective and standardized breast examinations in low-resource settings to detect breast lesions at early stages. The device can also be an effective screening tool for younger women with dense breasts.

KEYWORDS: iBreastExam, Breast cancer screening, India, Low-cost setting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-10

Title: Adjunct diagnosing with breast specific gamma imaging or ultrasound in symptomatic women with mammographic dense breasts: A single center retrospective and comparative analysis

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Body: Background: Mammographic density breasts decrease the diagnostic accuracy of mammography (MMG). While ultrasound (US) or breast specific gamma imaging (BSGI) can detect breast cancer in mammographic dense breasts, but these modalities have not been directly compared in prospective trials. We conducted a retrospective and comparative analysis of adjunct diagnosis to compare, within the same participants, by BSGI and US in mammographic dense breasts.

Methods: Diagnosing with BSGI or US in women with mammographic dense breasts is a retrospective and comparative study recruiting symptomatic women with mammographic dense breasts. Eligible women had performed BSGI and US with independent interpretation of adjunct imaging. Outcome measures included sensibility, specificity, and diagnostic accuracy (assessed by the area under the receiver operating characteristic curve) of combined MMG plus BSGI versus MMG plus US. These were compared using McNemar's test for paired binary data in this comparative analysis.

Results: From April 2013 to April 2016, 364 symptomatic and mammographic dense women (median age \pm SD, 51.12 \pm 10.92 years) recruited and had the final surgical pathology diagnosis, with 218 malignant diseases (59.89%) and 146 benign diseases (40.11%). Combined with BSGI or US can increase the sensibility, specificity and diagnostic accuracy of MMG. There is no difference for BSGI and US in enhancing the sensitivity of MMG diagnosis (Se-Difference: 3.21%, $P=0.23$), but the diagnosis specificity of MMG joint BSGI was more superior than MMG plus US (Sp-Difference: 10.27, $P=0.003$). ROC curve analysis of area under the curve showed that MMG plus BSGI has better diagnostic accuracy than MMG with US (0.90 vs. 0.83, $P=0.0019$). For different breast cancer molecular classification, MMG plus US seems more sensitivity for the Luminal A subtype than that of MMG plus BSGI, but without significant (Difference: 3.28, $P=0.687$). Other subtypes and the primary local tumor size of breast cancer did not affect the diagnostic sensitivity of MMG plus BSGI or US.

Conclusions: For the symptomatic women with mammographic dense breasts, MMG plus BSGI or US have similar diagnostic sensitivity, but the specificity and diagnostic accuracy of MMG plus BSGI was higher than MMG plus US. BSGI could potentially be the adjunct diagnosis modality in Chinese women with mammographic dense breasts.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-11

Title: Can ultrasound replace sentinel lymph node biopsy in breast cancer staging of the axilla? Results from a single center retrospective review of axillary evaluation with conventional sonography and ultrasound elastography

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Body: Background and objective

Sentinel lymph node biopsy (SLNB) is the gold standard for axillary staging in clinically node negative breast cancer patients. Although generally safe and accurate, SLNB is an additional surgical procedure with occasional instances of false negatives and complications arising. Hence, there is interest in finding less invasive alternatives to SLNB. The aim of the study is to determine if ultrasound (US), together with adjunct US elastography (EI), has the potential to replace SLNB by evaluating its overall accuracy as well as the nodal disease burden in false negative cases.

Materials and methods

Axillary US evaluations in breast cancer patients performed in a single center institution over a 7 year period (2009-2015) were reviewed. Lymph nodes with histological assessments from percutaneous or surgical biopsies were included in the study. Cases which had histology obtained only after neoadjuvant chemotherapy were excluded. US assessment was based on the retrospective review of the attending radiologists' US reports. EI was based on the elastographic size ratio calculated from available elastographic images obtained before US percutaneous biopsy of lymph nodes. The sensitivity, specificity and false negative rates were correlated with histology as the gold standard. For false negative cases, the number of nodes involved and the size of the largest metastatic focus were recorded from available pathology reports.

Results

608 axillary US studies performed on 578 patients with invasive breast cancers were reviewed. 254 (41.8%) cases had nodal metastases and 354 (58.2%) had benign histology. Overall US sensitivity, specificity and false negative rates were 74.8%, 91.0% and 25.2% respectively.

Comparison of the ultrasound evaluation of lymph nodes against the final nodal status after histological evaluation

		Ultrasound impression		
		Malignant	Benign	Total
Node status	Malignant	190 (74.8%)	64 (25.2%)	254
	Benign	32 (9.0%)	322 (91.0%)	354
Total		222	386	608

Of the 64 false negative cases, 81.3% (52/64) involved only 1 or 2 nodes, while false negative cases involving > 2 nodes constituted 4.7% (12/254) of all nodal metastases. 32.1%, 19.6%, 26.8% and 21.4% of the false negatives had a nodal metastatic size of 0.2-2mm, >2-5mm, >5-10mm and >10mm respectively (p=0.414). EI performed in 70 cases showed a sensitivity of 93.5% vs 97.8% (p=0.617) and specificity of 100% vs 58.3% (p<0.001) when compared against conventional US.

Comparison of the diagnostic accuracy between conventional US and EI

	US impression		EI impression		P-value
	malignant	Benign	Malignant	Benign	
Lymph node malignant (n=46)	45 (97.8%)	1 (2.2%)	43 (93.5%)	3 (6.5%)	0.617
Lymph node benign (n=24)	10 (41.7%)	14 (58.3%)	0 (0%)	24 (100%)	<0.001

Conclusion

US is fairly accurate in the evaluation of axillary nodal disease. Even in false negative cases, the nodal disease burden is low, potentially not affecting prognosis. Hence, axillary US staging has the potential to replace SLNB. Adding EI to conventional US evaluation will not significantly reduce the false negative rate but may help reduce unnecessary biopsies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-12

Title: Tomosynthesis as an alternative to magnetic resonance imaging (MRI) in assessing invasive lobular carcinoma (ILC) multifocality

Holt RE E, Topps A, Lim YY and Gandhi A. University Hospital of South Manchester NHS Trust, Manchester, United Kingdom and Manchester Academic Health Science Centre, Manchester, United Kingdom.

Body: Introduction

Invasive lobular carcinoma is associated with a higher rate of multifocality than other types of breast cancer. Breast MRI is commonly performed in patients diagnosed with ILC to assess for additional disease prior to formulating a management plan. MRI may be both time consuming and costly and can delay treatment.

Recently tomosynthesis has become an adjunct in the assessment and diagnosis of breast cancer. It is readily available at the time of mammography therefore providing information at the one stop clinic. We proposed that it may be possible to use tomosynthesis to assess for multifocality in ILC in place of MRI.

Method

A retrospective review of all cases of ILC diagnosed at a single regional screening unit over a 3-year period was performed. Patients having surgery as primary treatment were included. Ninety-eight patients were identified, 29 having both MRI and tomosynthesis in addition to mammography and ultrasound scan as part of their assessment. Histological data was used to compare the two imaging modalities. Bland and Altman limits of agreement analysis was performed to assess the difference between MRI and tomosynthesis in these patients. An acceptable difference (the difference in values that is unlikely to influence management) was set at 5mm.

Results

The Bland and Altman limits of agreement analysis produced three graphs to demonstrate the difference between MRI and Tomosynthesis, MRI and histology size and Tomosynthesis and histology size. It shows that the average difference between MRI and Tomosynthesis = 3.8mm (95% limits of agreement: -19.2 to 26.8mm). The percentage of 'acceptable' differences (defined as ≤ 5 mm) was 72% for MRI and tomosynthesis suggesting that there is good concordance between the two. Tumours measuring >30 mm on MRI are perceived as smaller under Tomosynthesis.

The analysis demonstrated the average difference between MRI and histology values is -1.3 mm (95% limits of agreement = -33.2 to 30.6) compared with an average difference between Tomosynthesis and histology of -5.1 mm (95% limits of agreement = -27.7 to 17.5). The percentage of 'acceptable' differences (defined as ≤ 5 mm) was 48% for both MRI vs histology and Tomosynthesis vs histology, suggesting that neither is superior when compared to final histology results.

In three patients there were multifocal cancers identified on histology that had not been detected by any imaging modality. In one patient there were MRI images that suggested a second tumour which was not present on histological examination. The same is true for a patient diagnosed with a second cancer identified by tomosynthesis but not found in the specimen. A third patient was diagnosed with a second tumour by both image modalities but again not present on histology. In one case tomosynthesis detected a true second cancer that was not identified on MRI.

Conclusion

We believe this preliminary study suggests that tomosynthesis is equivocal to MRI for assessing multifocality in ILC. If confirmed the implications would be a shorter investigative pathway with reduced delays and costs. A larger multi-centre trial to assess the use of tomosynthesis to replace MRI in patients with ILC is warranted.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-13

Title: Breast ultrasound surveillance with image fusion technique in a short-interval follow-up for BI-RADS category 3 mass lesions

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Body: **PURPOSE:** Breast ultrasound (US) is a diagnostic imaging modality that is now widely used as an adjunct to mammography for evaluation of breast abnormalities. In the Breast Imaging-Reporting and Data System (BI-RADS) lexicon, a solid mass with an oval shape, well-circumscribed margins and parallel orientation is classified as category 3 (BI-RADS category 3 mass lesions). This mass should have a risk of malignancy of < 2%. Surveillance breast US in short-interval follow-up for BI-RADS category 3 mass lesions is an acceptable alternative to biopsy. The surveillance US is recommended 6 month after initial lesion identification and then every year for at least 2 y. However, inconsistent reproducibility for US due to operator dependence is still a clinical issue. The aim of our study was to verify the utility of US using real-time virtual sonography (RVS) - to coordinate present US images with past US images reconstructed from previously acquired US volume data using magnetic tracking system – in a short-interval follow-up for BI-RADS category 3 mass lesions.

METHOD AND MATERIALS: We enrolled 20 women (23 lesions) with more than 24 months of follow-up after classification as BI-RADS category 3 during initial US. US surveillance was scheduled at 6, 12 and 24 months. Three-dimensional assessment of morphologic features was performed while serially checking past US volume data corresponding to the present US probe position. Measurement of the target lesion diameter was performed after the probe was adjusted to include the maximum diameter of a past US image at each visit.

RESULTS: RVS was technically successful in 100% of patients. All target lesions were detected, including two iso-echoic lesions. The mean target lesion diameters at baseline and at 6, 12 and 24 months were 8.2±4.2, 8.4±4.5, 8.1±4.5 and 8.3±5.0 mm, respectively. Statistical analysis using a Friedman test with multiple comparisons revealed no significant difference between the diameters at each time point ($p = 0.785$). RVS was used to directly compare the US morphologic characteristics and sized of lesions. Furthermore, the RVS data can be reproduced in their entirety for independent review at a later date by using stored US volume data.

CONCLUSION: Our results suggest that RVS is reproducible, operator-independent technique for comparison of US images of BI-RADS category 3 mass lesions obtained at different time points.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-02-14

Title: PD-1-IRDye800CW as a novel probe for fluorescence molecular imaging and image guided intraoperative surgery in breast tumor mouse model

Du Y, Chi C, Yang X and Tian J. Chinese Academy of Sciences, Institute of Automation, Chinese Academy of Sciences, Beijing, China.

Body: Background: Breast cancer, especially triple-negative breast cancer (TNBC), characterized by lack of expression of estrogen receptor (ER), progesterone receptor (PR), and HER2/neu (HER2), are generally high-grade, aggressive tumors with poorer disease-specific survival than other breast cancer subtypes. Therefore, novel imaging and therapeutic strategies are needed to improve the management of TNBC. Programmed cell death (PD-1) is an immune inhibitory receptor expressed by activated T cells that play a pivotal role in tumor immune evasion. Previous studies showed that intratumoral expression of T-cell inhibitory molecule PD-L1 was associated with high histologic grade and TNBC. Therefore, PD-1 was suggested to be an attractive target for antibody-guided fluorescent tumor imaging of breast tumor. **Aim:** The aim of this study is to create a novel near infrared fluorescence imaging probe PD-1-IRDye800 CW, which may facilitate a targeted *in vivo* fluorescence imaging and guide the intraoperative surgery of breast tumors. **Methods:** The 4T1 breast tumor bearing Balb/c mice were used in this study. PD-1-IRDye800CW probes were injected intravenously, and the ratIgG-IRDye800CW was used as control. The fluorescence imaging was performed at different time points including 0, 1 h, 4 h, 8 h, 12 h and 24 h after probe injection. And the surgery was performed 8 h post-injection with the aid of fluorescence imaging guided surgical navigation system. **Results:** It showed that PD-1-IRDye800CW reached the tumor site 30 min after tail vein injection. The fluorescence signal gradually accumulated at the tumor region 1 h to 8 h post-injection and then the tumor signal decreased 12 h thereafter. While the IgG-IRDye800CW control mice, the fluorescence signal can also be detected in the tumor region 30 min after injection, and then the signal was restricted to the tumor and kidney regions 1 to 4 h post-injection. Thereafter, most of probe was urinated, and the signal in the tumor regions decreased after 8 h. The fluorescence signal intensities of PD-1-IRDye800CW and IgG-IRDye 800CW probes at the tumor regions were compared under the same fluorescence scale bar, and we found that the PD-1-IRDye800CW probe showed a specific targeting and stable signal at the tumor region. To confirm the *in vivo* observation, the tumors and major organs were dissected out 8 h post-injection, and we found that the fluorescence signal of the PD-1-IRDye800CW probe in the tumors was stronger than the control probe. **Conclusion:** Our data suggested that through specific binding to the PD-1 expressing tumor infiltrating lymphocytes (TIL), the PD-1-IRDye800CW probe achieved a targeted imaging of breast tumors *in vivo*. Furthermore, the PD-1-IRDye800CW can work as an intraoperative imaging probe for the guidance of breast tumor surgery. These findings reinforce the potential of PD-1-IRDye800CW for the theranostic applications in clinics in the future.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-01

Title: The footprint of the aging stroma in older breast cancer patients

Brouwers B, Fumagalli D, Brohee S, Hatse S, Govaere O, Floris G, Van den Eynde K, Schoffski P, Smeets A, Neven P, Lambrechts D, Sotiriou C and Wildiers H. Laboratory of Experimental Oncology, KU Leuven & University Hospitals Leuven, Leuven Cancer Institute, Leuven, Belgium; Breast Cancer Translational Research Laboratory, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; University Hospitals Leuven, Leuven, Belgium; Multidisciplinary Breast Cancer, University Hospitals Leuven, Leuven, Belgium and Vesalius Research Center (VRC), VIB, KU Leuven, Leuven, Belgium.

Body: Introduction: Tumoral masses are not only composed of malignant cells, but also enclose a more or less ample stromal microenvironment, which has been shown to influence the cancer cell behaviour. As aging induces accumulation of senescent cells in the body, this microenvironment is thought to be different in cancers occurring in old patients compared to the younger counterparts. More specifically, senescence-related fibroblastic features, such as the Senescence Associated Secretory Profile (SASP) and the induction of Autophagy, are suspected to stimulate tumor growth and progression.

Materials and Methods: We compared gene expression profiles in stromal fields of breast carcinomas by performing laser capture microdissection of the cancer-associated stroma from 8 old (≥ 80 years at diagnosis) and 9 young (< 45 years at diagnosis) triple negative breast cancer patients. Gene expression data were obtained by microarray analysis (Affymetrix). Differential gene expression and Gene Set Enrichment Analysis (GSEA) were performed.

Results: Differential gene expression analysis showed higher growth-, dedifferentiation- and migration- promoting gene expression in the stromal samples of older vs younger patients. GSEA confirmed the presence of a SASP, as well as the presence of Autophagy in the stroma of older patients.

Conclusion: We provide the first evidence in humans that older age at diagnosis is associated with a different stromal microenvironment in breast cancers. The SASP and the presence of Autophagy appear to be important age-induced stromal features.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-02

Title: Stromal kinome screening identifies a novel regulatory kinase implicated in fibroblast-mediated progression of invasion in triple negative breast cancer tumours

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Body: Background:

The tumor microenvironment impacts the behaviour of surrounding epithelial cells and is thought to play a pivotal role in tumorigenesis while influencing treatment response. Still, the role of stromal cells-mediated paracrine signalling on tumor cells' fate and disease progression/remission remains largely unknown. Unravelling the significance of kinases in 'normal' stromal cells and deciphering the transmitted signals that result in reciprocal communications with tumor cells could lead to the identification of new combinatorial strategies for the treatment of breast cancer (BC). Considering the lack of effective therapies for triple negative BC patients (TNBC), we aimed to identify 'normal' fibroblasts-expressed kinases that modulate the invasive potential of TNBC tumor cells and characterise their mechanism(s) of action.

Methods / Results:

We performed a kinome siRNA screening in human mammary fibroblasts (HMFs). After silencing, HMFs were co-cultured with MDA-MB-231 TNBC cells using a 3D-matrigel assay, allowing cells to form spheroids. 3D cell invasion was measured at different time points using confocal microscopy (HMF and MDA-MB-231 were fluorescently labelled). Our screening revealed new kinases expressed in HMFs, whose inhibition was able to impede ('harmful' kinases) or promote ('protective' kinases) the invasion of MDA-MB-231 cells.

Mechanistic studies determined whether this effect was a result of direct interaction between cells, or if it was mediated via secretion of specific factors from stromal cells. Our data showed that the exosomes transmitted from normal fibroblasts (upon inhibition of the specific kinase) were responsible for the observed invasive phenotype of TNBC cells. Quantification/distribution of exosomes followed by mass-spectrometry determined the number, size and content of exosomes in the presence/absence of this kinase. Subsequent RNAseq and quantitative proteomics (SILAC) uncovered the alterations in signalling pathways and key proteins in TNBC cells, due to silencing of the specific kinase in normal fibroblasts.

Xenograft BC orthotopic studies further demonstrated that co-injection of TNBC cells with knock-out normal fibroblasts (for the kinase under study), results in reduced tumor growth (primary & metastatic) compared to co-injection of TNBC cells with wild-type normal fibroblasts.

In a clinical setting, we examined the expression (mRNA/protein) of the kinase of interest on TNBC patients consisting of primary normal and cancer associated fibroblasts (CAFs) (>5cm or <5cm from primary tumor site respectively). We also analysed various GSE datasets containing matched normal/CAFs samples along with patients' survival data and saw a reverse correlation of the expression of this kinase with overall and metastasis-free survival.

Conclusions:

Further understanding the biological complexity and contribution of the stromal cells in tumour development and expansion is of critical importance in our attempt to identify new putative targets for cancer treatment. Our work reveals a new 'undermining' kinase expressed in normal fibroblasts, inhibition of which can have beneficial effects for TNBC patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-03

Title: Microenvironment induced DDR2 mediates stromal-cancer interactions and metastasis growth in breast cancer

Kleer CG G, Martin EE E, Anwar T, Arellano-Garcia C, Lama A, Medhora N, Chen Y-C, Yoon E, Ge C, Franceschi R and Gonzalez ME E. University of Michigan, Ann Arbor, MI.

Body: Background: Accumulating evidence suggests that mesenchymal stem/stromal cells (MSCs) are recruited to the tumor microenvironment and play roles in tumor progression; however the underlying mechanisms by which MSCs promote breast cancer migration and invasion, and their role in metastasis need further investigation. Studies have demonstrated that collagen plays an important role in breast tumorigenesis by activating signaling pathways. We hypothesize that MSCs may promote breast cancer progression through regulating collagen-induced signaling in breast cancer cells.

Methods: We isolated carcinoma-associated MSCs (CA-MSCs) from human breast cancer metastasis to lymph node (LNM) and liver (LM). A portion of each specimen was stained with H&E to confirm the diagnosis, and a portion was mechanically dissected and digested with collagenase. Adherent CA-MSCs were subcultured in MSC medium for up to 12 passages. CA-MSCs were subjected to multilineage differentiation assays and labeled with Ds-Red. COL 1 expression and its receptor discoidin domain receptor 2 (DDR2) were downregulated in the CA-MSCs-DsRED using specific shRNA. We established single culture and co-cultures of CA-MSCs with GFP labeled breast cancer cells (BCCs) MDA-MB-231 and MCF10CA1a which were used for Live Imaging Microscopy, IHC, RT-PCR, WB, immunofluorescence, 3D proliferation and invasion assays, and *in vivo* xenograft experiments.

Results: CA-MSCs had spindle morphology, normal karyotype, were nontumorigenic *in vivo*, and possessed tri-lineage differentiation ability (osteoblast, adipocyte, and chondrocyte). CA-MSCs exhibited high mRNA and protein levels of collagen I (COL1) and its receptor DDR2. ShRNA-mediated knockdown of COL1 or DDR2 in CA-MSCs induced a change in morphology towards epithelial, decreased expression of epithelial to mesenchymal transition (EMT) markers, and impaired migration. Co-culture of CA-MSCs with BCCs led to increased BCC proliferation, EMT, invasion, and increased DDR2 expression in BCCs compared to single cultures of BCCs, which was blocked by COL1 and DDR2 shRNA in CA-MSCs. Live imaging studies revealed that shCOL1 and shDDR2 was sufficient to completely disrupt the organized migration pattern of BCCs aligned with CA-MSCs. *In vivo*, xenografts derived from MDA-MB-231 cells co-cultured with shControl CA-MSCs exhibited increased collagen I deposition in the tumor microenvironment, increased tumor growth, and metastasis compared to the single cultures of MDA-MB-231 cells. Remarkably, shDDR2 in CA-MSCs rescued the phenotype and reduced tumorigenesis and metastasis.

Conclusion: We successfully isolated and characterized CA-MSCs, confirming their presence in human breast cancer metastasis. Our findings suggest that collagen I and its receptor DDR2 play a role in directional migration of breast cancer cells in alignment with CA-MSCs, a function that may be implicated in breast cancer invasion and metastasis. Downregulation of DDR2 reduced tumor growth and metastasis *in vivo*. Modifying the tumor microenvironment by manipulating collagen I and/or DDR2 levels in MSCs might be therapeutically useful in preventing or halting metastasis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-04

Title: Tumor infiltrating macrophages, lymphocytes and matrix metalloproteinase 9 (MMP-9) expression in breast cancer

Pelekanou V, Brown JR R and Rimm DL L. Yale University, School of Medicine, New Haven, CT.

Body: Background: Immune therapy has been highly successful in tumors with high lymphocytic infiltrate, but they only represent the minority of breast neoplasms. Macrophages rather than lymphocytes, are more prominent in mammary development and disease. Specific markers of breast tumor associated macrophages (TAMs) remain to be defined. Local interactions define their plasticity and activity, rendering in situ investigation important in their characterization. MMP-9 is an important regulator of breast cancer microenvironment that could mediate cross-talk between TAMs and tumor cells. Here we objectively measure CD68 and CD163 and also MMP-9 within each macrophage subtype to determine the relationship between macrophage expression, tumor infiltrating lymphocytes (TILs) and molecular subtypes in breast cancer.

Methods: Using a multiplexed quantitative immunofluorescence (QIF)-based assay for simultaneous detection of DAPI (all cells), Cytokeratin (epithelial cells, clone CK8/CK18), CD163 (M2 Macrophages, clone CD163-L-U), CD68 (pan macrophage marker, clone PG-M1), and MMP-9 (Matrix Metalloproteinase 9, clone D6O3H XP). We measured the levels of protein expression in breast carcinomas on two sets of Yale tissue microarrays (TMA) [YTMA201 (all breast cases, n=399) and YTMA149 (Triple Negative, n=160)]. Markers were measured using the AQUA method of QIF on TMAs at two-fold redundancy. Linear regression coefficients (R^2) were used to compare antibody QIF scores within cores from different areas of the tumors. Median cut-point was used to stratify patients for overall and disease specific survival (OS and DSS).

Results: Cases with high TILs, as shown by assessment of CD3, 8 and 20, generally show an inverse relationship with both CD68 and CD163, especially in ER+ cases. MMP-9 was then measured in both subtypes of macrophages. In ER+ tumors MMP-9 was expressed in CD163+ macrophages ($p=0.007$), while in TNBC it was found in CD68+/CD163- macrophages ($p<0.001$). In all cases MMP-9 was significantly higher in ER- cases (CD68+/CD163- $p=0.0001$), (CD163+ $p=0.01$). In ER+ cases high MMP-9 expression was associated with shorter OS ($p<0.0001$ in CD163+ cells). On the contrary, in TNBC high MMP-9 was associated improved DSS in the CD68 compartment ($p=0.007$).

Discussion: Using an objective, quantitative multiplex assay for synchronous measurement in tumor and microenvironment, we found an inverse relationship between TILs and macrophage infiltration, suggesting immune modulation by different cellular elements. Within the macrophage population, we found that MMP9 expression is a function of the breast cancer molecular phenotype. Most significantly, the ER status of the tumor is correlated with the macrophage subtypes that express MMP9. Efforts to determine the clinical value of these observations are underway to better determine the balance between pro- and antitumor immunity in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-05

Title: Role of stromal discoidin domain receptor-1 in breast cancer

Gupta K, Yuan B and Li R. University of Texas Health Science Center, San Antonio, TX.

Body: Breast cancer is highly heterogeneous and associated with a myriad of risk factors. Women with high mammographic density have 2-6 fold higher risk of developing breast cancer and changes of tumor stroma are predictive of patient survival. Also, obesity at diagnosis is linked to 33% increase in risk of overall mortality in women with early-stage breast cancer. However, the cause for poor prognosis in obesity associated breast cancer is not fully understood. Studies show mechanical forces in the breast stroma as key regulators of mammary tumorigenesis. These mechanical cues are transduced through discoidin domain receptor-1 (DDR-1), which forms part of the arsenal of cell surface receptors that mediate cell-collagen interactions. DDR-1 expression is upregulated in breast cancer-associated stroma in several preclinical tumor models and has been linked with poor prognosis. However, little is known about how stromal DDR-1 contributes to tumorigenesis. **Therefore, I aim to investigate the functions of DDR-1 in the tumor stroma of the breast** and its precise role in breast cancer. **Findings:** To address this, orthotopic injection of mesenchymal like-mouse mammary tumor cells (M-Wnt) were done in DDR-1 knockout (KO) mice and wild type (WT) mice, and tumor growth was monitored. I observed a significant reduction in tumor growth in KO mice vs WT. Immunohistochemistry of the tumors showed reduced expression of collagen-1A in KO. Given the association of obesity with cancer, I generated a cohort of High Fat diet (HFD) induced obese KO and WT mice for tumor studies. I observed enhanced tumor growth in both WT-HFD and KO-HFD mice vs the non-obese mice. Moreover, percent difference in tumor weight from KO and WT obese mice was higher than that from non-obese. To elucidate the causative factors, circulating plasma proteins were assessed for cancer biomarkers in these cohorts. Out of the 20 analytes checked, circulating Endothelin-1 (ET-1) was significantly increased in WT non-obese mice. ET-1 also positively correlated with the tumor volume measurements in these mice. Recent studies show ET-1 to be a pro-fibrotic factor. This is in line with the increased expression of Col-1A in WT tumors. Intriguingly, I did not observe an increase in plasma ET-1 in the obese WT mice. There were overall reduced circulating Leptin, IL-6 and G-CSF levels in KO-non obese, and this difference was more pronounced in KO-obese. G-CSF levels were significantly high in WT-HFD vs KO-HFD. To access the tumor microenvironment, RNA-Seq was done on tumors obtained from the above two cohorts. My RNA seq data confirmed abrogation of both ET-1 and IL-1 β / IL-6 signaling axis in the KO. Additionally, RNA -Seq revealed significant upregulation of T-cell activation gene signatures in the KO mice. Immune suppressive TGF- β signaling genes were up-regulated in WT. High ECM protein gene expression in the WT tumors could contribute to reduce immune cell infiltration and immune suppression. **Summary:** Host DDR-1 plays a definitive role in breast tumorigenesis. Increased Endothelin-1, ECM proteins, and immune suppression in the WT tumors are responsible for their enhanced tumorigenesis. The host DDR-1 dependent tumorigenesis gets further aggravated in case of diet induced obesity in ET-1 independent manner and, by enhanced immune-suppression.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-06

Title: Microenvironmental control of breast cancer subtype elicited by paracrine growth factors signaling

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Body: During cancer progression from an indolent stage to a clinically manifested disease, the surrounding microenvironment co-evolves into an activated state through continuous and dynamic paracrine communication between all constituent cell types that collectively sustain malignant growth. Hence, from a therapeutic perspective, tumors must be regarded as multi-cellular organs. Mesenchymal cells are highly plastic entities. Normal fibroblasts are described to counteract tumorigenesis by repressing cancer initiation. However, co-evolution of the malignant epithelium and its underlying stroma instigates activation of cancer-associated fibroblasts (CAFs) into a phenotype endorsing most, if not all, hallmarks of cancer progression. Through translational studies using breast cancer patient cohorts, genetically engineered mouse models (including patient derived xenografts), and single-cell RNA sequencing, we delineate a previously unappreciated role for CAFs as determinants of breast cancer prognosis and treatment response through specification of the molecular subtype of breast cancer. Expression of paracrine growth factors mediating malignant cell-CAF crosstalk in breast cancer was found to be associated with a hormone receptor-negative state. Genetic targeting of the signaling networks governing the interactions between tumor cells and CAFs confirmed their functional importance by prompting a delay in tumor growth, impaired extra-cellular matrix deposition and reduced angiogenesis. Importantly, our work holds therapeutic implications, as genetic or therapeutic targeting of CAF subsets resulted in conversion of basal-like/triple-negative breast tumors into a luminal state that conferred sensitivity to endocrine therapy in previously impervious tumors. Additionally, to mechanistically explore the heterogeneity of mesenchymal cells in breast tumors, we have made use of single cell RNA sequencing of isolated CAFs as a guide to divide cells into transcriptionally similar groups. Distinct clusters of different CAF subtypes were identified and found to denote diverse functions in the tumor microenvironment. In conclusion, therapeutic targeting of functional subsets of CAFs may be exploited as a refined strategy to improve patient benefit from established treatments by impinging on the stimulatory crosstalk between CAFs and their surrounding malignant microenvironment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-07

Title: Inhibition of cancer-associated fibroblast function by farnesoid X receptor activation: Experimental basis for a novel therapeutic strategy in breast cancer

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Body: Background: Breast cancers are heterogeneous tissues comprised of multiple components, including tumor cells and microenvironment cells. Fibroblasts are the most abundant cell type in tumor-associated stroma (named as Cancer Associated Fibroblasts, CAFs), with multiple roles in tumor initiation and promotion through soluble secreted factors, extracellular matrix proteins, or direct cell-cell contacts. While the significance of CAFs is well recognized, the treatment of CAFs in clinical practice has not yet been established. Our previous studies have shown that activation of the nuclear Farnesoid X Receptor (FXR) in mammary epithelial cancer cells resulted in reduced proliferation, migration, and invasion 'in vitro' and tumor growth 'in vivo'. Here, we have investigated the role of FXR in CAFs to evaluate whether this receptor may affect their tumor-promoting features.

Methods: Human CAFs were isolated from biopsies of primary breast tumors (n=4) and characterized for expression of CAF markers. Immortalized CAFs were also used. FXR expression was evaluated by realtime RT-PCR, immunofluorescence and immunoblotting analyses. To assess the functional role of FXR in CAFs, we tested the effects of the synthetic FXR agonist GW4064 by using MTT proliferation, wound healing and Boyden chamber-based transmigration assays. RNA sequencing was used to compare the transcriptomes of vehicle- and GW4064-treated CAFs. Ingenuity Pathway Analysis (IPA) was used to calculate the activation z-score of molecular canonical pathways. The impact of FXR activation on tumor-promoting abilities of CAFs was tested in coculture experiments with human estrogen receptor -positive MCF-7 and -negative SKBR-3 breast cancer cells.

Results: We evidenced, for the first time, FXR mRNA and protein expression in CAFs. GW4064 treatment did not affect proliferation of CAFs; in contrast, it was able to significantly decrease their motility. Quantitative transcriptome profiling of CAFs treated or not with GW4064 revealed 1182 differentially expressed genes, of which 285 were upregulated and 330 were downregulated (fold change ≥ 1.5) in response to GW treatment. In line with our previous experiments, IPA evidenced a marked reduction in the activity of RhoA signaling, signaling by Rho Family GTPases, regulation of actin-based motility by Rho showing activation z-score of -1, -0.83, and -0.82, respectively. A decreased Rho A GTPases signaling profile is consistent with the reduced migration capabilities of GW4064-treated CAFs, as these proteins are known to govern cell cytoskeleton organization, migration, and metastasis dissemination. Finally, MCF-7 and SKBR-3 breast cancer cells cocultured with GW4064-treated CAFs showed reduced anchorage-independent growth, suggesting how GW4064 exposure may also influence secretion of tumor-promoting soluble factors from CAFs.

Conclusions: The present and our previous studies propose FXR ligands as potential pharmacological tools that, targeting both cancer and activated stromal cells, may represent a more effective approach to treat breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-08

Title: Mechanisms of CD8+ T cell immunosuppression in triple negative breast cancer

Gruosso T, Gigoux M, Bertos N, Zuo D, Manem V, Monette A, Lapointe R, Haibe-Kains B and Park M. Goodman Cancer Research Center, McGill University; Princess Margaret Cancer Centre, University Health Network and Laboratoire d'Immuno-Oncologie, ICM, Université de Montréal/CHUM Research Center (CRCHUM).

Body: Triple negative breast cancer (TNBC), defined as tumors lacking expression of the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), are especially difficult to treat effectively. While ER+ and HER2+ breast cancer subtypes can be treated with Tamoxifen and Herceptin, respectively, there are no targeted therapies for TNBC patients. Furthermore, while only 20-30% of TNBC patients respond to chemotherapy in the neoadjuvant setting, overall outcome remains poor for non-responding patients. However, mounting evidence suggests that immune-checkpoint inhibitor immunotherapies may be especially promising for TNBC patients. We and others have shown that the presence of CD8+ T cells, a crucial component of the cytotoxic arm of the adaptive immune response, is a sign of good clinical outcome in TNBC patients. However, good outcome only correlates with CD8 +T cell invasion of the tumor parenchyma. Some patients had an accumulation of CD8+ T cells in the surrounding tumor-associated stroma, but not the tumor epithelia, and these patients responded as poorly as patients with no CD8 T cells at all. Yet how cancer associated fibroblasts (CAFs), the dominant cell type of the tumor-associated stroma, affects CD8+ T cell invasion into the tumor epithelia is still poorly understood. To identify potential stroma-dependent mechanisms which potentiate or inhibit CD8+ T cells invasion into the tumor epithelia, we performed gene expression profiling of laser-capture microdissected tumor-associated stroma (and matched epithelia) from 38 TNBC cases. Here we identify several stromal and epithelial canonical pathways as well as biomarkers that are associated with and may explain the accumulation of CD8 T cells outside of the tumor epithelia.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-09

Title: Human breast cancer biopsies induce eosinophil recruitment and enhance adjacent cancer cell proliferation

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Body: Previous research has demonstrated that chronic inflammation facilitates cancer progression and spread to other sites. The effect of acute inflammation in the microenvironment of the tumor that develops as a result of standard biopsy procedures has not been studied in detail. Recent studies demonstrated that acute inflammation in mouse models from a mammary biopsy increases the frequency of distal cancer metastasis. Core needle biopsies are the standard procedure for breast cancer diagnosis, but there have been no studies detailing the inflammatory response from the biopsy. The purpose of this study is to determine if core needle biopsies in breast cancer patients trigger an inflammatory response, determine the type of inflammatory response that occurs and study the potential effect of acute inflammatory response on tumor cells remaining. The biopsy wound site was identified in primary tumor resection samples from breast cancer patients. The inflammatory response adjacent and distant to the biopsy were studied via histology and immunohistochemistry. Tumor cell proliferation was studied as well. Our study demonstrated that diagnostic core needle biopsies trigger inflammatory cell recruitment at the site, and those cells remain over long periods of time. An unexpected increase in eosinophils were found to accumulate at the biopsy site. The biopsy also was shown to increase proliferation adjacent to the wound site. Overall, core needle biopsies used for breast cancer diagnosis induce a unique inflammatory environment at the site of the biopsy as well as the closely surrounding cells. Inflammation induced by the biopsy could lead to tumor cell progression or metastasis in breast cancer. These findings may be important for the clinical management of breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-10

Title: Prognostic and predictive marker in the tumor microenvironment of breast cancers

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Body: Background: Though genetic alterations initiates' tumor formation, extracellular signals from the tumor microenvironment can modify the growth and spread of cancer cells. We are studying a small modifier protein, tissue inhibitor of metalloproteinase-4 (TIMP-4) that can accelerate tumor growth and metastatic potential as seen in both cell culture, animal experiments and prospective studies of clinical subjects. TIMP-4 is synthesized by stromal cells and secreted into the tumor microenvironment. It binds to the membrane-bound tetraspanin CD63 on tumor epithelial cells and induces the activation of the tumor promoting PI3K/AKT pathway. This modifier has its strongest effect on the so-called triple-negative breast cancers (TNBC) and breast cancers with HER2 gene amplification.

Methods: Cell culture experiments using the human MDA-MB-468 (TNBC) and SK-BR-3 (HER2 amplified) cell-lines were used to assess the effects of elevated levels of TIMP-4 on downstream targets. Cells with or without human recombinant TIMP-4 added to the growth medium were used to determine the effects on growth, AKT activation, apoptosis and response to anti-TIMP-4 treatment. Tumor growth in nude mice with or without TIMP-4 containing slow-release pellets implanted into the mammary fatpad was followed for six-week period to assess response to anti-TIMP-4 therapy. Lungs, liver, spleen and mammary fatpads were collected and analyzed. Prospectively collected patient samples, in accordance with the IRB approved protocol, were tested for circulating levels of TIMP-4 and the cancer marker CA15-3, using commercially available ELISA kits, in samples collected prior to chemotherapy and at each treatment cycle.

Results: Augmentation of TIMP-4 levels in the cell culture medium of TNBC and HER2-amplified cells resulted in accelerated growth and increased activation of AKT as determined by Western blotting for pAKT^{Ser 473} and pAKT^{Thr 308}. Adding an anti-TIMP4 therapeutic antibody to TNBC cells grown in the presence of TIMP-4 resulted in reduced growth. HER2-positive cells grown under identical condition and treated with either an anti-TIMP-4 or a neutralizing ErbB2 antibody demonstrated no effects on growth. Only dual blockade of HER2 and TIMP-4 resulted in reduced growth. Using the anti-TIMP-4 therapy in animals resulted in stable disease with no signs of metastatic growths. Patients undergoing treatment for TNBC or HER2-positive breast cancer that had continuous elevated TIMP-4 levels while on treatment had a higher recurrence rate than patients with diminishing TIMP-4 levels in response to therapy. The TIMP-4 levels preceded and correlated better with disease progression than the traditional CA15-3 marker.

Conclusion: Together these clinical and laboratory data suggests that TIMP-4 could be a prognostic as well as a predictive marker. Our preliminary clinical data indicates that TIMP-4 might be a more reliable marker for recurrence/progression than CA15-3. Also, our cell and animal studies indicate that TIMP-4 could be therapeutic target for TNBC and HER2-positive breast cancer patients that test positive for this marker. Thus providing a targeted therapy for TNBC patients and HER2-positive patients that do not have full clinical benefit of HER2-targeted therapies alone.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-11

Title: Fibroblasts isolated from the “normal-like” tissue adjacent to breast tumours suppress healthy epithelial progenitor cell proliferation while supporting tumour cell growth

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Body: The tissue adjacent to breast tumors has been referred to as “normal-like” tissue despite exhibiting many alterations at epigenetic and gene expression levels consistent with enhanced proliferation and wound healing signatures. However, the influence of such alterations on the proliferation and differentiation of healthy breast progenitors is currently unknown. Fibroblasts are a major component of microenvironment for the healthy and malignant breast cells. We therefore, isolate fibroblast from primary breast tumors, tissue adjacent to tumors (TAT) and the healthy breast tissue and examine their ability to support proliferation of healthy and malignant breast cells. To characterize the TAT samples we first utilized clonal co-culture assays using breast cells obtained from the healthy breast tissue (reduction mammoplasty sample, RM) and the healthy fibroblasts. Our results suggested that the TAT samples surprisingly contained significantly decreased pool of progenitors compared to the RM samples. In order to study the underlying mechanism, we characterized fibroblasts derived from either the breast tumours (TAFs) or the TAT samples (TATF) or the RM normal samples (NAFs) and assessed their role on breast progenitor cell functions. Fibroblasts were isolated from the ER+ and ER- breast tumours and their adjacent breast tissue. We observed that matrigel co-cultures consisting of RM samples and NAFs led to a 5.5-fold expansion of the progenitors, whereas the co-cultures of TAT or the RM samples with either TAFs or TATFs failed to show expansion of epithelial progenitors. The comparative secretome analysis of the NAFs and the TATFs identified TGF β as a candidate molecule primarily secreted only by the TATFs and not by NAFs. Interestingly, blocking TGF β signaling restored both TAFs' and TATFs' ability to support the expansion of healthy progenitors in matrigel cultures. Lastly, we found that TAFs were able to enhanced breast cancer cell proliferation *in vivo* and *in vitro* but to a lesser extent than the TAFs. Our observations suggest that the tissues adjacent to breast tumours are transformed into a TGF β -enriched environment that is supportive of breast tumour growth while suppressing the proliferation and differentiation potentials of the healthy breast progenitors. Our data also suggest that the use of TGF β blockers may be important in reducing risk of local breast tumour recurrence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-12

Title: Cancer-associated fibroblasts release exosomal microRNAs that dictate an aggressive phenotype in breast cancer

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Body: Cancer-associated fibroblasts (CAFs) are the major components of the tumor microenvironment. They may drive tumor progression, although the mechanisms involved are still poorly understood. Exosomes have emerged as important mediators of intercellular communication in cancer. They mediate horizontal transfer of microRNAs (miRs), mRNAs and proteins, thus affecting breast cancer progression. Differential expression profile analysis identified three miRs (miRs -21, -378e, and -143) increased in exosomes from CAFs as compared from normal fibroblasts. Immunofluorescence indicated that exosomes may be transferred from CAFs to breast cancer cells, releasing their cargo miRs. Breast cancer cells (BT549, MDA-MB-231, and T47D lines) exposed to CAF exosomes or transfected with those miRs exhibited a significant increased capacity to form mammospheres, increased stem cell and epithelial-mesenchymal transition (EMT) markers, and anchorage-independent cell growth. These effects were reverted by transfection with anti-miRs. Similarly to CAF exosomes, normal fibroblast exosomes transfected with miRs -21, -378e, and -143 promoted the stemness and EMT phenotype of breast cancer cells. Thus, we provided evidence for the first time of the role of CAF exosomes and their miRs in the induction of the stemness and EMT phenotype in different breast cancer cell lines. Indeed, CAFs strongly promote the development of an aggressive breast cancer cell phenotype.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-13

Title: Obesity and breast cancer: The adipocyte-macrophage interaction as a mediator of metastasis

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Body: Obese individuals with breast cancer have a poorer prognosis and higher risk of metastatic disease vs. non-obese patients. Prior research has largely focused on defining the interactions between adipocytes and tumor cells, but other cell types may also play a role. Adipose tissue in obese individuals is characterized by a significant infiltration of macrophages. We have therefore investigated the adipocyte-macrophage interaction as a possible mechanism whereby obesity promotes breast cancer metastasis.

We performed co-culture with both human and murine cells to determine whether adipocytes influence the expression of metastasis-promoting signals in macrophages. For the human system we co-cultured primary breast adipocytes with THP-1 macrophages. For the murine system we co-cultured 3T3-L1 differentiated adipocytes with J774.1 macrophages. We then assayed the mRNA levels and protein expression of metastasis-promoting genes.

In both systems we found that co-culture with adipocytes increased the expression of Vascular Endothelial Growth Factor A (VEGF-A) in macrophages. In examining a potential mechanism, we also show that media collected from cultured adipocytes (adipocyte-conditioned media) activates the transcription factor Egr-1 in macrophages. Since Egr-1 is a known regulator of VEGF-A expression, this is a potential mechanism whereby VEGF-A transcription is up-regulated. Treatment of human macrophages with Interleukin-6, Leptin and Insulin induced VEGF-A levels to a similar degree as co-culture, identifying these as adipocyte-derived signals that can possibly mediate this effect.

In summary we have found that the interaction between adipocytes and macrophages leads to the up-regulation of the pro-angiogenic signal VEGF-A in macrophages. Therefore this represents a potential mechanism whereby obesity could promote breast cancer metastasis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-14

Title: Stem cell and macrophage markers are enriched in normal tissue adjacent to inflammatory breast cancer

Reddy JP P, Atkinson RL L, Larson RA A, Burks JK K, Smith D, Debeb BG G, Ruffell B, Creighton C, Reuben JM M, Krishnamurthy S, Symmans WF F, Brewster A and Van Laere SJ J. MD Anderson Cancer Center, Houston, TX; University of South Florida, Tampa, FL; Baylor College of Medicine, Houston, TX and University of Antwerp, Belgium.

Body: Introduction: We hypothesized that normal breast tissue in inflammatory breast cancer (IBC) patients contains intrinsic differences, including increased mammary stem cells and macrophage infiltration, which may promote the IBC phenotype.

Materials and Methods: Normal breast tissue at least 5cm away from primary tumors were obtained from mastectomy specimens. This included an initial cohort of 8 IBC patients and 60 non-IBC patients followed by a validation cohort of 19 IBC patients and 25 non-IBC patients. Samples were immunostained for either CD44⁺CD49f⁺CD133/2⁺ stem cell markers or the CD68 macrophage marker and correlated with IBC status. Automated quantitation of positive cells was employed for the validation cohort. We also examined the association between IBC status and previously published tumorigenic stem cell and IBC tumor signatures in the validation cohort samples.

Results: 8 of 8 IBC normal tissue samples expressed CD44⁺CD49f⁺CD133/2⁺ stem cell markers in the initial cohort as opposed to 0/60 non-IBC normal tissue samples ($p=0.001$). Similarly, the median number of CD44⁺CD49f⁺CD133/2⁺ cells was 25.7 in the IBC validation cohort as opposed to 14.2 in the non-IBC validation cohort ($p=0.007$). 7 of 8 IBC samples expressed CD68⁺ macrophages in initial cohort as opposed to 12/48 non-IBC samples ($p=0.001$). In the validation cohort the median number of CD68⁺ cells was 3.7 in the IBC cohort vs 1.0 in the non-IBC cohort ($p=0.06$). Normal tissue of IBC patients was positively associated with a tumorigenic stem cell signature ($p=0.02$) and with a 79-gene IBC gene signature ($p<0.001$).

Conclusions: Normal tissue from IBC patients is enriched for both mammary stem cells and macrophages. Further, normal tissue of IBC patients has higher association with both a tumorigenic stem cell signature and IBC-specific tumor signature. Collectively, these data suggest that normal tissue from IBC patients is distinct from non-IBC normal tissue and may support the hypothesis that a primed normal breast contributes to the development of IBC symptoms upon oncogenic insult. Validation of these results in additional normal tissue in cancer-free women would better determine causality.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-15

Title: Joint influence by paracrine adipocyte signals and metabolic conditions on breast cancer

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Body: Background: The growing global population of overweight and obese people represents a pressing public health concern. A higher percentage of body fat is linked to an altered metabolic state and an increased risk of chronic metabolic disorders and cancer. Although, the mechanistic explanations for how obesity affects the development, progression and prognosis of cancer is incompletely mapped.

Purpose: To investigate the joint influence by adipocytes and metabolic pressure on breast cancer cell expansion.

Experimental Design: *In vitro* differentiated 3T3-L1 adipocytes were exposed to metabolic glucose^{low/high} and insulin^{low/high} pressure in order to mimic normal, pre-diabetic, overt diabetic and late diabetic conditions. Adipocyte-conditioned medium was collected and paracrine effects by adipocyte-derived factors on estrogen receptor (ER)-positive (T47D, MCF-7) and ER-negative (MDA-MB-231) breast cancer cell proliferation, molecular adaptations, and cell motility were subsequently analyzed using sulforhodamine B, Western immunoblotting and migration assays.

Results: Under normal metabolic conditions, adipocytes stimulated the growth of ER+ (1.1-2.0-fold; $P < 0.001$) and to a greater extent, ER- breast cancer cells (3.1-fold; $P < 0.001$), compared with controls. The joint effects by adipocyte paracrine signals and higher metabolic pressure (overt or late diabetic conditions), further enhanced the proliferative response in both ER+ and ER- cells (1.3-3.5-fold; $P < 0.01$), compared with controls. Furthermore, adipocyte-derived factors induced morphological changes, protrusion extensions and cell migration in the low invasive T47D cells. Additional acquisition of molecular epithelial-mesenchymal transition features were observed in breast cancer cells following co-culturing with adipocyte-derived factors.

Conclusion: These study results support the hypothesis that paracrine signals by adipocytes significantly stimulate the proliferation and induction of a more motile phenotype of human breast cancer cells, which is further enhanced under obesity-associated metabolic conditions.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-16

Title: FAP α (fibroblast activation protein- α) analysis in breast tumor cells and stroma after neoadjuvant treatment

Calvo I, Prieto M, Suárez-Gauthier A, Pérez FJ J, Hernández E, Acosta D, Cárdenas JM M, López-Ríos F and Estévez LG G. Breast Cancer Unit of Centro Integral Oncológico Clara Campal, Madrid, Spain; IT – Information Technology. San Pablo University, Madrid, Spain and Medicine Faculty. San Pablo CEU University, Madrid, Spain.

Body: Background: FAP α is a transmembrane serine protease expressed on cancer associated fibroblast that promotes tumour growth and invasion. In patients (pts) with poor outcome and survival FAP α is highly overexpressed. FAP α is also expressed in stroma across all breast cancer subtypes without association with clinicopathological factors. Pts without Complete Pathological Response (pCR) after Neoadjuvant Chemotherapy (NC) had poor outcome. We analysed the relationship between the expression of FAP α in stroma (fibroblast) and in epithelial breast cancer cells of pts without pCR after NC (taxanes, anthracyclines and trastuzumab in Her2+).

Methods: 60 pts were included. ER, PR and Ki67 were studied by IHQ (Ventana) and Her2 by FISH (PatnVysion). FAP α expression was determined by IHQ (polyclonal, Ventana). St Gallen guidelines for subtype of breast cancer were used.

Results: 53 pts had tissue. Median age 47 years (range 29-68). Median tumour size 43mm and 10 (18.9%) were multifocal. 37 (69.8%) had positive axillary nodes. 47 were ductal invasive carcinomas. 33 (62.3%) were grade 2 and 20 (37.7%) grade 3. 44 pts (83%) had ER+ (20 luminal B), 17 (32%) Her2+ and 6 (11%) triple negative(TN). Median Ki67 was 22% (p25-75:15-38%). Miller-Payne response was 1.9% G1 (1pt), 43.4% G2 (23 pts), 41.5% G3 (22 pts) and 13.2% G4 (7 pts). The recurrences were 2 local and 12 distant (26.4%). Median FAP α in residual epithelial breast cancer cells after NC was 85% (p25-75:30-95%) and in the stroma 20% (p25-75:10-62%). Median epithelial FAP α was 55% in TN, 85% Her2, 72.5% luminal A and 92.5% in luminal B. Median stromal FAP α was 52.5% in TN, 20% Her2, 15% luminal A and 15% in luminal B. There is not association between stromal FAP α and clinicopathological features, but a higher epithelial FAP α was found in tumours with higher ER, PR and Ki67. In luminal B subtype, stromal FAP α was smaller in pts with relapses (median 7.5%) than without relapses (median 30%).

Conclusions: Stromal FAP α in residual cancer after NC is higher in TN breast cancer but without association with relapses in our small sample. However, in luminal B subtype stromal FAP α is smaller in pts with relapses.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-17

Title: Tartrate-resistant acid phosphatase (TRACP) modulates breast cancer pre-metastatic niche

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Body: Background: Tartrate-resistant acid phosphatase (TRACP or ACP5) is a metalloprotein enzyme that belongs to the acid phosphatase family and is mainly expressed by osteoclasts. It is a classic marker for bone resorption and osteoclast differentiation. TRACP expression is a useful serum marker for extensive bone metastasis. Recent studies have reported that TRACP promotes the invasion and distant metastasis of melanoma and breast cancer cells through the modulation of focal adhesion kinase (FAK) phosphorylation and epithelial cell migration. Therefore, TRACP has received considerable attention as a newly discovered proinvasion metastasis driver associated with different malignancies. Additionally, TRACP overexpression is indicative of the poor prognoses cancer patients and suggests that TRACP may have an important role in promoting tumor metastasis. Its detail mechanism in modulating cancer metastasis remains unknown. We have previously demonstrated the serum TRCAP 5a is a valuable marker in assessing disease activity, severity or systemic macrophage (inflammatory) burden. It has recently been found that up-regulation of TRACP promoted cancer cell invasion and lung metastasis, whereas TRACP knockdown inhibited these processes.

Aims: No studies have previously addressed the question of extracellular effects of TRAP. The aim of this study was to evaluate if TRACP binds to and is endocytosed by cancer cells. We also aim to determine if extracellular TRACP affect oncogenesis or cancer progression, alter the cancer cell expressions of angiogenesis or adhesion molecules, or direct the treatment resistance or cancer prognosis.

Results: Knockdown of TRACP significantly attenuated FoxM1-enhanced invasion and lung metastasis. Extracellular TRACP in culture media influenced the tumor invasion upon endocytosis. Significant increased invasion/migration was observed upon higher concentrations of TRACP. Tumor growth was significantly enhanced when the tumor xenografting previously primed with polarized M2 \emptyset or TRACP protein, by enhanced metalloproteinase (MMP-9) and angiogenic factor (VEGF-A) expression

Conclusions: TRACP or M2 \emptyset -secreted TRACP could promote cancer growth and modulated the tissue and microenvironment and create pre-metastatic condition by cell adhesion/angiogenesis signaling alteration. TRACP-targeting strategy is plausible in breast cancer metastasis treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-03-18

Title: A hierarchy of cancer associated fibroblasts in situ and in circulation promote breast cancer metastasis

Sharma U, Miller P, Speransky S, Medina-Saenz K, Ferrer P, Lippman M and El-Ashry D. University of Miami, Miami, FL.

Body: Background: Metastasis is the primary cause of breast cancer mortality. Interactions between cancer cells and non-cancer cells of the tumor microenvironment (TME) are pivotal in governing tumor initiation, progression and metastasis, and cancer associated fibroblasts (CAFs) are critical orchestrators of these interactions. We recently identified circulating CAFs (cCAFs) as a novel circulating biomarker associated with metastatic breast cancer. We established CAF cell lines from dissociated luminal A, ER- Her-2 amplified, and triple-negative/basal-like (TN) breast tumors. We demonstrated that “aggressive” CAFs differentially secrete miRNAs that contribute to ER-negativity, activated growth factor signaling, and induction of EMT in breast cancers compared to “indolent” CAFs. We hypothesized that a hierarchy exists within CAFs regarding their ability to facilitate tumor progression and metastasis. Here we demonstrate that CAFs derived from aggressive TN breast tumors differ from those derived from more indolent Luminal A breast tumors in secretion of cytokines and chemokines that can confer differential effects on the behavior of breast cancer cells. We also demonstrate that “aggressive” CAFs more potently facilitate tumor progression and metastasis than “indolent” CAFs. We additionally evaluated if “aggressive” and “indolent” CAFs differ in their ability to mobilize CTCs and circulating CAFs into circulation.

Methods: Conditioned media (CM) from “aggressive” and “indolent” CAFs was analyzed for chemokine/cytokine expression. Luminal A breast cancer cells (MCF-7) or primary tumor cells from an aggressive TN tumor (DT28) were injected into the mammary fat pad of 6-8 week old female NSG mice, either alone or in combination with CAF19-I or CAF23-A. Tumor progression was monitored and mice were examined for metastasis at necropsy. Tissues were harvested for histology and blood was collected by cardiac puncture. Plasma was analyzed for cytokine/chemokine expression and blood was processed for enumeration of circulating tumor cells (CTCs) and cCAFs.

Results: “Aggressive” CAF CM had significantly higher levels of a number of factors, including IL-8, SDF-1, and CXCL1, compared to “indolent” CAF CM. MCF-7 cells co-injected with “aggressive” CAFs formed tumors much faster than those co-injected with the “indolent” CAFs or without CAFs. While DT28 cells readily form tumors and metastasize in the NSG model, fewer DT28 cells do not form metastases in the timeframe that this same lower number of DT28 cells co-injected with “aggressive” CAFs demonstrated robust tumor growth and developed metastases in liver and pancreas. DT28 cells co-injected with “indolent” CAFs did not exhibit metastases.

Conclusion: The data presented here further demonstrate that there is a hierarchy within CAFs regarding their ability to facilitate tumor growth and metastasis, and that this may largely be mediated by secreted soluble factors. “Aggressive” CAFs may retain their programmed role in circulation and accelerate metastasis more than “indolent” CAFs. We suggest that targeting CAFs in situ and in circulation and disrupting their interactions with breast cancer cells could provide novel strategies to combat breast cancer and breast cancer metastasis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-04-01

Title: A paradigm shift for insulin-like growth factor-1 receptor function in triple negative breast cancers

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Body: Loss of insulin growth factor (IGF) signaling in the mammary gland at puberty leads to delayed elongation and defects in terminal end bud (TEB) proliferation. Moreover, IGF signaling is essential for epithelial mesenchymal interactions in mammary bud morphogenesis. We have generated and characterized a mouse line containing both *MMTV-Wnt1* and *MMTV-dnIGF-1R* (kinase dead-*IGF-1R*) transgenes (referred to subsequently as bigenic mice) that leads to increased mammary tumor initiation and metastasis¹. Recent analyses of TCGA human datasets support the relevance of our model in human triple negative breast cancers (TNBCs) that have reduced IGF-1R expression and increased expression of Wnt1 and the Frizzled co-receptor Lrp6. We have identified alterations in a number of target genes that potentially regulate metastasis in the bigenic primary tumors including increases in interleukin-6 (IL-6), Nanog, Slug, and decreases in E-cadherin expression as assessed by Qiagen specific qPCR and single gene qPCR. Furthermore, a subset of anti-inflammatory chemokines and cytokines were assessed by a targeted qPCR array revealing increases in interleukin-4, interleukin-10, and C-C motif chemokine ligand-2 and decreases in tumor necrosis factor-alpha and interleukin-1 in the bigenic tumors suggesting a tumor associated macrophage phenotype. By flow cytometry analysis, the ratio of CD4+ T cells to CD8+ T cells is increased in the bigenic tumors suggesting a tumor promoting immune microenvironment. However, increased expression of IL-6 in the bigenic tumors is specific to the luminal tumor cells, and inhibition of IGF-1R in the MCF7 human breast cancer cell line results in increases in both IL-6 and Slug expression suggesting the tumor cells activate EMT and a pro-tumorigenic microenvironment in response to decreased IGF-1R. Taken together, our data suggest IGF-1R acts as a negative regulator of mediators that promote invasion and metastasis in TNBCs.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P4-04-02

Title: Bromodomain and extra-terminal proteins regulate the epithelial-mesenchymal transition in breast cancer

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Body: BACKGROUND. The epithelial-mesenchymal transition (EMT) is a developmental program that cancer cells often activate to acquire a highly plastic phenotype that promotes invasion, metastasis, but also chemoresistance and cancer stem cell generation. As readers of epigenetic marks, bromodomain and extra-terminal (BET) proteins BRD2, BRD3 and BRD4 participate in the regulation of multiple transcriptional programs implicated in cancer progression. We sought to unravel the roles of BET proteins in EMT in breast cancer.

METHODS. Cell line models of the different subtypes of breast cancer were used in this study (luminal A: MCF-7, T47D, basal-like: MDA-MB-231, SUM149PT). BET expression was modulated by using RNA interference or plasmid-mediated overexpression. Small molecule JQ1 was used to inhibit BET proteins. The expression of 84 key EMT genes was monitored by PCR using a Qiagen kit. We also monitored EMT by measuring the expression of epithelial and mesenchymal markers by immunoblot and immunofluorescence staining.

RESULTS. Despite their homology, we report that BET proteins differentially regulate EMT. Based on an EMT PCR array, we identified a BRD2-specific transcriptional profile that promotes EMT, whereas BRD3 and BRD4 signatures repress this program. These individual signatures are unidentifiable upon pan-BET inhibition using JQ1, reinforcing the necessity to target each BET member separately to better understand their functions. Upon BRD2 depletion, basal-like breast cancer cells, which present a mesenchymal phenotype, exhibit a reduced expression of mesenchymal markers (N-cadherin, vimentin) and re-express epithelial markers (E-cadherin, cytokeratins). Moreover, a large panel of EMT master transcription factors is downregulated in BRD2-depleted cells, including the Snail and ZEB families or Twist. Interestingly, we found that BRD3 or BRD4 depletion leads to the opposite phenotype: an increase of mesenchymal marker expression and repression of the epithelial markers. In luminal A breast cancer cells which present an epithelial phenotype, BRD2 overexpression leads to the expression of mesenchymal markers. Similar results were obtained by depleting BRD3 or BRD4 in these cells, confirming the differential roles of BET proteins in EMT regulation.

CONCLUSION. Taken together, our results establish that BRD2 positively regulates EMT, whereas BRD3 and BRD4 repress this program. BET proteins possess separate and opposite biological functions, reinforcing the relevance of an individual targeting instead of a pan-BET inhibition using JQ1. We hypothesize that BET proteins modulate EMT through the regulation of its master transcription factors. We propose that the balance of BET proteins present at the promoters of the EMT genes is a novel mechanism of regulation of this program in breast cancer cells.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-04-03

Title: Obesity-induced EMT in luminal A breast cancer cells

Hayden A, Quach D, Galvan G, Patodia R, Brenner A and deGraffenried L. The University of Texas at Austin, Austin, TX and The UT Health Science Center at San Antonio, San Antonio, TX.

Body: Introduction: Obesity is associated with a worse prognosis in breast cancer, including the less aggressive ER+ luminal A subtype, but the mechanisms by which it promotes disease progression are unclear, making treatment difficult. Obese breast cancer patients have a higher risk of a more aggressive disease compared to lean patients, which is associated with treatment resistance and metastasis. The mechanisms promoting obesity-driven metastasis are not understood, but several studies have indicated that obesity is associated with a “stem-like” phenotype. A “reprogramming” occurs, transforming stationary, epithelial cells to motile, malignant cells that exhibit a more aggressive phenotype than their stromal counterparts. Our data suggest that *in vitro* exposure of luminal A breast cancer cells to obese conditions may induce an epithelial to mesenchymal transition (EMT), which is characterized by a more stem-like phenotype, resistance to treatment (chemo, hormone and radiation), as well as greater metastatic potential. This has let us to hypothesize that one critical mechanism by which obesity promotes a more aggressive disease is through inducing an EMT reprogramming, resulting in a more stem-like phenotype.

Methods and Results: Both *in vitro* and translational approaches will be done to determine if obesity induces epigenetic reprogramming associated with a more stem-like phenotype. MCF-7 ER+ breast cancer cells exposed to 2% sera from obese (BMI ≥ 30) postmenopausal women demonstrated a significant increase in expression of both SNAIL1 and TWIST transcription factors (9-fold and 4-fold, respectively) which are implicated in EMT and potentially stem-cell programming, compared to those exposed to sera from lean women. Current studies are underway to determine if this is observed in other ER+ luminal A cell lines, including T47D, and whether induced changes in these transcription factors results in changes in signaling pathways associated with EMT, including TGF β , which can activate the PI3K–AKT, ERK MAPK, p38 MAPK and JNK pathways and WNT signaling, which promotes EMT by stabilizing β -catenin. Additionally, the luminal A cell lines will be assessed for changes in other factors known to modulate breast cancer cell programming, including KLF4, OCT4, SOX2, and NANOG.

Conclusions: Our earlier studies have demonstrated that obesity promotes a more aggressive disease even in luminal A disease. The mechanisms for this remain unclear. Our exciting preliminary findings suggest that obesity might induce a reprogramming of the luminal A well-differentiated cell to a more stem-like phenotype. Our results will lay an important foundation for understanding how obesity modulates breast cancer disease progression, whether this programming may provide therapeutic target to improve response and overall survival in the obese patient.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-04-04

Title: Eribulin impairs TGF- β type I receptor localization and signaling in BT-549 cells

Kaul R, Risinger AL L and Mooberry SL L. University of Texas Health Science Center at San Antonio, San Antonio, TX and Cancer Therapy & Research Center at San Antonio, San Antonio, TX.

Body: Microtubule targeting agents (MTAs) continue to be some of the most valuable drugs used to treat breast cancer. While decades of research have shown that these drugs cause mitotic arrest in cells by suppressing the dynamic instability of microtubules, recent evidence demonstrates that the ability of MTAs to disrupt microtubule-dependent transport of key signaling components, including proteins and microRNAs, in interphase cells likely contributes to their anticancer actions. TGF- β signaling is a driver of oncogenesis and epithelial-to-mesenchymal transition (EMT) that involves multiple protein trafficking and intracellular signaling events. Ligand mediated activation of the cell surface TGF- β receptors leads to downstream signaling in the canonical and the non-canonical pathways that collectively lead to the expression of proteins implicated in EMT, including Snail and Slug. Additionally, TGF- β type 1 receptor (TGF- β R1) undergoes constant cycling from the plasma membrane to the cytosol, a microtubule-dependent process. We tested the hypothesis that a short-term treatment of breast cancer cells with eribulin or 3 other clinically relevant MTAs would differentially disrupt interphase microtubules and alter the transport and downstream signaling of TGF- β R1. BT-549 cells were treated for 2 h with concentrations of MTAs that cause comparable disruption of the interphase microtubule network; 100 nM was used for the destabilizers, eribulin or vinorelbine and 1 μ M was used for the stabilizers, paclitaxel or ixabepilone. The results show that TGF- β R1 was extensively localized along the stabilizer-induced microtubule bundles, a phenomenon not observed with destabilizer-treated cells. The downstream consequences were further assessed. The expression of Snail and Slug were evaluated in cells pretreated with MTAs followed by TGF- β stimulation. Eribulin and vinorelbine significantly inhibited the TGF- β -induced expression of Snail and Slug while stabilizers did not alter their expression levels. We hypothesize that eribulin and vinorelbine are inhibiting the non-canonical TGF- β signaling pathway since they had no effect on the localization and expression of Smad2/3, proteins involved in the canonical pathway. Eribulin induced inhibition of TGF- β signaling is consistent with previous studies that show that a 7 day treatment reversed TGF- β mediated EMT in breast cancer cells¹. Data from patients shows that eribulin decreases the plasma concentration of TGF- β within 7 days of treatment initiation². Our data suggest that eribulin rapidly inhibits non-canonical TGF- β signaling indicating that this is a potential mechanism for the eribulin-mediated EMT reversal. This information, together with previously published reports, suggest that eribulin has multiple effects leading to inhibition of TGF- β signaling. These studies begin to shed light into the diverse mechanisms of action of MTAs.

1. Yoshida T, et al., *Brit J Cancer* 110(6): 1497-505, 2014

2. Ueda S, et al., *Brit J Cancer*, 2016

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2016 San Antonio Breast Cancer Symposium

Publication Number: P4-05-01

Title: Diet, development and predisposition to breast cancer: The impact of sugar derived metabolites (AGEs) on pubertal mammary gland development

Krisanits BA A, Nogueira LM M, Findlay VJ J and Turner DP P. Medical University of South Carolina, Charleston, SC.

Body: The mammary gland is one of the few organs that continues to develop postnatally through stages including puberty, pregnancy, lactation, and involution. The gland is composed of epithelial and stromal cells that include fibroblasts, adipocytes, endothelial cells, nerve cells, and macrophages. Terminal end bud (TEB) structures are found exclusively in the pubertal developmental stage. The formation of TEBs and side branching drives mammary gland epithelial cell invasion into the mammary fat pad, continuing until the entire fat pad is filled. Pubertal mammary gland morphogenesis integrates a balance of epithelial cell proliferation, differentiation, and apoptosis. Several studies have shown that the interaction between mammary epithelial and stromal cells is crucial for the proper postnatal development of the mammary ductal tree. Interestingly, studies have shown that processes important in mammary gland development are often deregulated during breast cancer tumorigenesis. Thus, understanding the complex signaling network as well as the interactions between the different cell types during mammary gland development will be vital for elucidating the mechanisms underlying breast cancer progression and metastasis.

Glycation is the non-enzymatic glycosylation of sugar moieties to biological macromolecules such as protein and DNA which produces reactive metabolites known as advanced glycation end products (AGE's). AGE content in the Western Diet has consistently increased over the last 50 years due to increased consumption of sugar laden and cheap processed/manufactured foods which are high in reactive AGE metabolites. AGE containing food can lead to the accumulation of AGEs in the body overtime leading to pro-inflammatory and pro-oxidant effects when signaling through receptor for advanced glycation end products (RAGE). Leading too many complications associated with diseases including diabetes, Alzheimer's, heart disease and cancer. Preliminary data in our lab has shown that AGEs also have an effect on phosphorylation and signaling of estrogen receptor α ($ER\alpha$), a key receptor and signaling pathway in the regulation of mammary gland development during puberty. This observation, together with the links between diet, mammary gland development and immune cell recruitment lead us to examine the biological effects of a diet high in AGEs on pubertal mammary gland development in mice. We observed a significant disruption of normal pubertal mammary gland development in mice fed a high AGE diet when compared to mice fed a control diet. Mice fed the high AGE diet showed increases in TEB number as well as width, length and area. We also observed an increase in ductal branching and a decrease in ductal extension. Future studies will assess the role of macrophage recruitment to the developing gland, specifically around the TEBs based on its reported role in normal TEB function. We also plan to assess $ER\alpha$ signaling in mice fed the high AGE diet based on the reported role of estrogen signaling in ductal elongation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-05-02

Title: Conditional inactivation of the 25-hydroxyvitamin D-24-hydroxylase (Cyp24a1) in the mouse mammary epithelium alters mammary gland development

Sheng L, Turner AG G, Tarulli GA A, Barratt K, Kremer R, Morris HA A, Callen DF F and Anderson PH H. Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia; Dame Roma Mitchell Cancer Research Laboratories (DRMCRL), Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia; School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia and McGill University Health Centre, Montreal, QC, Canada.

Body: The biologically active form of vitamin D ($1,25(\text{OH})_2\text{D}$) regulates proliferation, differentiation, and apoptosis in diverse cell types. We have previously identified anti-proliferative activities of $1,25(\text{OH})_2\text{D}$ in human breast tissue, as well as $1,25(\text{OH})_2\text{D}$ target gene expression consistent with reports that elevated vitamin D levels may protect against cancer. In mouse studies, vitamin D signaling modulates normal mammary gland development, including ductal outgrowth and branching, and protects against tumorigenesis. Degradation of $1,25(\text{OH})_2\text{D}$ is initiated by the enzyme Cyp24a1 in target tissues, providing critical local control of $1,25(\text{OH})_2\text{D}$ bioactivity. *In vitro*, blockade of Cyp24a1 activity potentiates the anti-proliferative effects of $1,25(\text{OH})_2\text{D}$. However, the extent to which endogenous Cyp24a1 activity within the mammary epithelium regulates local $1,25(\text{OH})_2\text{D}$ levels to modulate normal mammary gland development, with possible implications for cancer, has not been investigated. We generated a novel mouse model with conditional knockout of the *Cyp24a1* gene specifically in the mammary epithelium (MMTV-Cre x *Cyp24a1*^{lox/lox}). Ablation of Cyp24a1 activity in the mammary epithelium does not alter either gland or body weight at 4, 6 or 10 weeks of age. Preliminary analyses of mammary gland whole mounts indicate that virgin knockout mice form fewer terminal end buds compared to glands from wild-type littermates at 4 and 6 weeks of age ($P < 0.05$). Moreover, the width of the ducts proximal to the central lymph node of knockout mice was less than that of wild-type mice at 4 and 10 weeks of age ($P < 0.05$ and $P < 0.01$, respectively). In addition, the number of secondary and tertiary branching points is reduced in mammary glands from knockout mice at 6 weeks of age ($P < 0.05$ and $P < 0.01$, respectively). In summary, our findings suggest that Cyp24a1 activity within epithelial cells plays a crucial role to modulate postnatal mammary gland development, presumably by limiting the local accumulation of $1,25(\text{OH})_2\text{D}$.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-06-01

Title: Patient derived DCIS mouse-intraductal (MIND) models recapitulate the full spectrum of human patient pathology and histologic features including progression to invasion in a subset of cases

Behbod F, Limback D, Hong Y, Elsarraj H, Berger J, Heddens E, Valdez K, Smith WP P, Inciardi M, Reddick M, Gatewood J, Darrah J, Winblad O, Meierotto R, Ricci L, Wagner J, Amin A, May L, Cusick T, Mammen J, Fields T, Godwin AK K, Fabian C, Kaufman C, Tawfik O and Fan F. University of Kansas Medical Center, Kansas City, KS; Truman Hospital, Kansas City, MO; St. Luke's Hospital, Kansas City, MO and Wichita School of Medicine, Wichita, KS.

Body: Introduction: The 20-year breast cancer mortality rate following a DCIS diagnosis is ~3%. Radiation and anti-hormonal therapy of DCIS has not resulted in improved overall survival, which argues against the non-selective use of such therapies in DCIS management. However, some DCIS cases do progress to invasive cancer, and these patients may benefit from treatment. A study of 80 DCIS patients followed for up to 30 years reported a 43% progression rate. Notably, progression was not predicted by grade, as 39% of even low-grade DCIS ultimately progressed to invasive cancer. Clearly, there is a need to identify which DCIS lesions are likely to progress. We have developed a novel mouse xenograft model (mouse-intraductal; MIND) to study the molecular basis of DCIS progression and enable identification of suitable biomarkers that predict invasive progression.

Methods: MIND involves injection of epithelial cells derived from patient breast lesions into the mammary ducts of immunocompromised mice. Serial sections of mouse mammary glands containing DCIS xenograft lesions were examined at time intervals of 3-14 months post-engraftment by histology using hematoxylin and eosin (H&E) and immunohistochemistry using anti-human cytokeratin 5/19, smooth muscle actin, ER, PR, p53, Ki67 and HER-2.

Results: Intraductal injection of cells derived from breast lesions of 28 patients into 133 mice resulted in a successful engraftment rate of 60%. Among these, 12 pure DCIS samples were injected into 35 different mice to create MIND xenografts. As early as three months post-engraftment, the DCIS xenograft cells showed multilayered *in situ* growth consisting of atypical neoplastic cells with prominent and vesicular nuclei. DCIS MIND xenografts exhibited the full spectrum of human DCIS histologic features, including similar biomarker expression (ER, PR, Ki67, HER-2 and p53) at long-term follow-up after engraftment (up to 12 months). Most remarkably, a subset of xenografts representing 5 patients (5/12; 42%) showed progression to invasion 6-12 months post-engraftment in the absence of any external genetic manipulations. This rate is very similar to that reported for human DCIS progression in untreated patients. MIND DCIS xenograft lesions that progressed showed disruption of basement membrane and myoepithelial layer by the invasive cells, retraction of basement membrane, and micro-invasion. MIND DCIS lesions were enriched in small capillaries, and in some cases clusters of invasive cells appeared inside nearby blood vessels.

Conclusion: The MIND xenograft is a viable model for human DCIS progression that recapitulates histologic features of human DCIS, as well as reported rates of progression to invasion. The availability of this innovative model provides a valuable tool for the discovery of new biomarkers to identify DCIS with invasive potential. The identification of high risk DCIS will ultimately help patients and clinicians choose the best course of therapy and avoid the morbidity and costs associated with unnecessary treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-06-02

Title: The mammary ducts create a favourable microenvironment for xenografting of luminal and molecular apocrine breast tumours

Bonnefoi H, Richard E, Grellety T, Velasco V, MacGrogan G and Iggo R. Bergonie Cancer Institute, INSERM U1218, University of Bordeaux, Bordeaux, France.

Body: There is a paucity of models for hormone receptor positive (HR+) breast cancer because of the difficulty of establishing xenografts from these tumours. We show that this barrier can be overcome by injecting human tumour cells directly into the mammary ducts of immunodeficient mice. Tumours from 31 patients were infected overnight with a lentiviral vector expressing tdTomato and injected through the nipple into the mammary ducts of NOD-SCID-IL2RG^{-/-} mice. Tumours formed in the mice in 77% of cases after the first injection (6/8 luminal A; 15/20 luminal B and 3/3 molecular apocrine). Four luminal A and one molecular apocrine graft were tested in secondary and tertiary grafts: all were successfully passaged in secondary and 4/5 in tertiary grafts. None of the samples engrafted when injected subcutaneously. The morphology, oestrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR) and Ki-67 profiles of the clinical samples were maintained in the tertiary grafts. We also show that the intraductal approach can be used to test the response to targeted therapy with fulvestrant and palbociclib, using a genetically defined ER+ model. Fulvestrant was ineffective in this model but palbociclib inhibited tumour growth and prevented conversion of DCIS into IDC. We conclude that the mammary ducts create a microenvironment that is uniquely favourable to the survival and growth of tumours derived from mammary hormone-sensing cells. This approach opens the door to testing genomically-targeted treatment of HR+ tumours in precision medicine programs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-06-03

Title: An annotated collection of pre- and post-therapy breast cancer patient-derived xenograft models built from fine needle aspiration samples aligned with ongoing clinical trials documenting response to treatment

Echeverria GV V, Chang JT T, Cai S, Tu Y, McCoy A, Lau R, Redwood A, Kaffiabasabadi S, Rauch GM M, Adrada BE E, Jennifer L, Moulder SL L, Symmans WF F and Piwnica-Worms H. M.D. Anderson Cancer Center, Houston, TX; University of Texas Health Science Center, Houston, TX; M.D. Anderson Cancer Center, Houston, TX; M.D. Anderson Cancer Center, Houston, TX and M.D. Anderson Cancer Center, Houston, TX.

Body: BACKGROUND: Patient-derived xenograft (PDX) models of breast cancer replicate the diverse histologic and molecular features of patient tumors and provide a renewable source of human tumor tissue; however collection of tissue by core needle biopsy is problematic due to patient discomfort, bleeding risk and the limited number of passes a patient can tolerate. In addition, FDA guidelines caution that multiple core needle biopsies could lead to an overestimation of the true pCR rate in neoadjuvant trials.

METHODS: To support the neoadjuvant molecular diagnostic and drug development program in TNBC, a pilot study was conducted to determine if fine needle aspiration (FNA) could be used for building PDX models. Prior to engraftment, FNA samples were analysed for cell number and viability.

RESULTS: Six PDX models were successfully generated from eight individual tumor samples. These models retain histologic and molecular features of the original patient tumors as determined by immunohistochemistry, RNA expression profiling, and deep whole-exome and targeted gene sequencing. In addition, the tested PDX models recapitulate the responses to therapies across multiple chemotherapeutic agents.

Based on this success, we have standardized the use of FNAs to generate PDX models both pre- and post-therapy in two ongoing neoadjuvant clinical trials:

1. MDACC 2014-0185 (PI Stacy Moulder, 360 patients), 'Improving outcomes in TNBC using molecular triaging and diagnostic imaging to guide neoadjuvant therapy'
2. MDACC 2014-0045 (PI Jennifer Litton, 20+ patients), 'A pilot study of BMN673 as a neoadjuvant study in patients with a diagnosis of invasive breast cancer and a deleterious BRCA mutation'

	FNA cells (x10 ⁴)	Cell viability (%)	Total viable cells (x10 ⁴)
Study entry biopsy (n=67)	144.50	50.65	44.14
Post treatment biopsy (n=16)	47.07	32.54	28.38

To date, treatment-naïve primary tumor samples from 67 patients enrolled onto these neoadjuvant trials, and 16 matched non-responsive post treatment tumor samples have been analysed for cell count and viability (table below) prior to being engrafted into the humanized mammary fat pads of NOD/SCID mice.

CONCLUSION: We have demonstrated success in using FNAs to build PDX models that recapitulate the biology and clinical course of the original tumor. In our pilot study, we successfully generated six PDX models using FNA for TNBC, including some harboring deleterious BRCA1/2 mutations. Because of the high concordance in histologic, genomic, and clinical attributes, we are now using this approach to develop a rich resource of pre- and post-treatment PDX models for the investigation of therapeutic resistance.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-06-04

Title: Triple negative breast cancer patient-derived xenografts: Molecular characteristics and sensitivity to emerging therapies

Evans KW W, Yuca E, Akcakanat A, Scott SM M, Paez Arango N, Zheng X, Chen K, Tapia C, Tarco E, Eterovic AK K, Black DM M, Jennifer LK K, Tripathy D, Mills GB B and Meric-Bernstam F. M.D. Anderson Cancer Center, Houston, TX.

Body: Background: Patients with triple negative breast cancer (TNBC) that have significant residual disease following neoadjuvant therapy have a poorer prognosis. The difficulty in developing improved treatment strategies for these patients is compounded by the molecular diversity of this pathology. Thus, preclinical efficacy testing with models representing the molecular heterogeneity of TNBC may not only improve efficacy testing but also has the potential to facilitate biomarker discovery to inform patient selection.

Methods: We generated 28 hormone receptor^{-low}/HER2⁻ patient-derived xenografts (PDXs) from 26 patients; the majority from residual disease following neoadjuvant chemotherapy. We characterized the tumors from the patients and the matched PDXs by targeted exome sequencing, RNA sequencing, and Reverse Phase Protein Arrays. We established single agent sensitivity for a set of PDXs to standard TNBC therapies as well as emerging targeted therapies targeting PARP, mTOR, PI3K and MEK1/2.

Results: PDXs maintained DNA/RNA profiles similar to the patients' tumors. Our PDX set exhibits a diverse set of potential driver alterations and is comprised of PDXs representative of all TNBC molecular subtypes. The PDXs varied in their sensitivity to standard chemotherapeutics. PI3K, mTOR and MEK inhibition caused tumor growth inhibition in several PDX models but were unable to cause tumor regression as single agent. In contrast, talazoparib caused dramatic regression in 5 out of 12 PDXs tested, including 3 PDXs from patients whose tumors had progressed on neoadjuvant anthracyclines and/or paclitaxel. Four of these talazoparib sensitive PDXs were derived from patients not harboring BRCA1/2 germline alterations; three had somatic alterations in genes linked to DNA damage repair (ATM, BRCA2, PTEN). The comparison of PARP sensitivity with molecular profiles will be presented.

Conclusions: TNBC PDXs represent molecular tumor heterogeneity as well as heterogeneity in response to targeted therapeutics. PDXs represent an opportunity for biomarker discovery as well as for rationale combination therapies to enhance the efficacy targeted therapies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-06-05

Title: Treatment of *ESR1* mutant and *PIK3CA* mutant patient-derived breast cancer xenograft models reveals differential anti-tumor responses to estrogen receptor degraders and PI3K inhibitors *in vivo*

Young A, Crocker L, Cheng E, Lacap J, Hamilton P, Oeh J, Ingalla E, Arrazate A, Hager J, Nannini M, Friedman L, Daemen A, Giltneane J and Sampath D. Genentech, Inc. and Seragon Pharmaceuticals, Inc..

Body: The phosphoinositide 3-kinase (PI3K) pathway is a key driver of hormone receptor (HR)-positive breast cancer growth and survival. It is estimated that 40-45% of HR+ breast cancers harbor oncogenic mutations in the *PIK3CA* gene, which encodes the p110 α isoform of PI3K. Tasisib (GDC-0032) is a mutant-selective PI3K inhibitor that demonstrates enhanced potency in *PIK3CA* mutant breast cancer cells and is being developed as a treatment for metastatic breast cancer that targets *PIK3CA*-mutant, HR-positive, HER2-negative patients. Activating mutations in the *ESR1* gene were recently described in metastatic breast cancer. These mutations confer hormone independent growth and may be associated with resistance to aromatase inhibitors. Drugs that selectively bind and antagonize the Estrogen Receptor alpha (ER α) protein and target it for degradation, such as fulvestrant, are referred to as selective estrogen receptor degraders (SERDs). Preclinical activity of the orally bioavailable SERD, GDC-0810, has not been well characterized in *ESR1* mutant PDX models. Therefore, our aim was to evaluate the efficacy and pharmacodynamic responses to agents that target ER α and PI3K as monotherapies and in combination, in *ESR1* and *PIK3CA* mutant HR+ breast cancer patient-derived xenograft (PDX) models. We hypothesized that mutational status of *ESR1* and *PIK3CA* may predict the responsiveness of HR+ PDX models to SERDs and PI3K inhibitors *in vivo*. Characterization of seven PDX models included authentication of hormone receptor status by immunohistochemistry (IHC) and determination of *ESR1* and *PIK3CA* genotype and allele frequency by exome sequencing. For a subset of models that utilize estrogen for growth, mice were supplemented with 17 β -estradiol, and cells or tumor fragments were implanted into the fat pad of intact female NOD-SCID or NOD-SCID-IL2Rgamma null mice and treated with fulvestrant, GDC-0810, or tasisib. Both fulvestrant and GDC-0810 were efficacious in *ESR1* wild type (WT) and mutant PDX models but to variable degrees ranging from tumor stasis to growth delay, with GDC-0810 resulting in superior single agent activity at relevant clinical exposure in the WHIM20 and WHIM43 *ESR1* mutant models. *PIK3CA* mutations (E542K, E545K, M1004V, and H1047R) were confirmed in six PDX models and PI3K pathway activation verified by strong pS6RP IHC staining. Tasisib induced tumor growth inhibition and tumor regressions in models harboring *PIK3CA* mutations, and models with no detectable expression of WT p110 α were the most sensitive. In the WHIM43 (*ESR1* D538G, *PIK3CA* M1004V), HCI-011 (*ESR1* WT, *PIK3CA* E545K) and HCI-013 (*ESR1* Y537S, *PIK3CA* H1047R) PDX models, combining fulvestrant and tasisib treatment further enhanced tumor growth inhibition with respect to either treatment alone. Our studies demonstrate the diverse anti-tumor responses of HR+ PDX models to SERDs and the PI3K inhibitor tasisib in the context of clinically relevant *ESR1* and *PIK3CA* mutations. Pharmacological and genomic characterization of additional PDX models may aid in strengthening associations between genotype, drug sensitivity and predictive biomarkers of response.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-06-06

Title: Consistent dosing-time dependent tolerability of everolimus (EV) in a pilot study in women with metastatic breast cancers (MBC) and in a mouse chronopharmacology investigation

Giacchetti S, Li XM, Ozturk N, Cuvier C, Machowiak J, Arrondeau J, Chang-Marchand Y, Espié M, Okyar A and Lévi F. Breast Disease Unit, Hopital Saint Louis, Assistance Publique-Hopitaux de Paris (AP-HP), Paris, France; 1INSERM U935, Team "Cancers Chronotherapy and Optimization of Hepatic Function", Villejuif, France; Université Paris-Sud, Orsay, Villejuif, France; 3Istanbul University, Faculty of Pharmacy, Beyazit-Istambul, Turkey; University Paris Diderot, Paris 7, Paris, France and Cancer Research Unit, Warwick Medical School, Coventry, United Kingdom.

Body: Background

The molecular circadian clock regulates mTOR activity, the main target of EV. EV use is hampered by adverse events in MBC patients (pts). Knowing the optimal timing of EV oral intake could improve tolerability, thus compliance and treatment efficacy.

Objective: To assess the relevance of EV timing for side effects in MBC pts, and to determine the impact of EV timing for body weight, circadian rest-activity pattern, and liver circadian clock in mice.

Materials and methods:

From 2013 to 2014, 19 MBC pts were treated with EV 10mg plus hormonotherapy at St Louis hospital Breast Cancer unit. Morning or Evening oral intake was prescribed by the medical oncologist in charge of each patient for both EV and hormonotherapy. Side effects were compared according to EV timing. We further investigated whether oral (po) EV timing mattered mice regarding tolerability. EV (5-20 mg/kg/day x 14 days) was administered through oral gavage at 4 circadian times, 6 hours apart to different groups of synchronized male or female B6D2F1 mice (n=32/sex). Body weight change and lethal toxicity were the endpoints. EV effects were further determined on clock gene *Per2* expression in the liver of *Per2::luciferase* (*Per2::luc*) mice. *Per2::luc* expression was monitored in freely-moving mice every minute for 3 days before, 5 days during and 3 days after daily EV at one of two timepoints using Real Time-Biolumicorder (RT-BIO, Geneva, Sw). Periods and amplitudes of *Per2::luc* rhythms were determined with spectral analysis.

Results:

Nine pts took EV in the morning and 10 in the evening for a median duration of 7.5 months (1.3-19.2 m). Median age was 59 (39-85), median delay between metastatic disease and EV treatment was 52.5 months. Pts had a median of 3 prior lines of treatment for MBC. EV was associated with exemestane (13 pts), or tamoxifen (6 pts). Evening EV was associated with higher occurrence of edema (6 vs 2 pts), fatigue (9 vs 2 pts), and pneumonia (3 vs 0 pts). Equitoxic doses of EV were twice as high in female as compared to male mice. EV timing at the beginning of the light (rest) span resulted in a lethal toxicity rate of 25% in female mice receiving 20 mg/kg/d and 50% in male mice dosed with 10 mg/kg/d). In contrast all the mice treated with EV at the beginning of the dark (activity) span survived, irrespective of dose level. Maximum body weight loss ranged from 5% following EV timing near activity onset to 12% after EV delivery near rest onset, both in males (5 mg/kg/d) and females (10 mg/kg/d) (ANOVA p<0.001). The circadian expression of liver *Per2::luc* was abolished following EV dosing near rest onset. *Per2::luc* remained rhythmic in mice receiving EV at the beginning of the activity span.

Conclusion: Tolerability was largely improved by the delivery of EV near the onset of the activity span both in women with MBC and in male or female mice. Morning administration of EV could minimize metabolic alterations and fatigue in humans. This timing should further avoid circadian disruption in the liver molecular clocks thus ensure better treatments tolerability.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-06-07

Title: Fibroblast growth factor receptor activation and breast tumor progression in a mouse xenograft model of obesity

Wellberg EA A, Anderson SM M, Jacobsen BM M, Johnson S, Kabos P, Sartorius C and MacLean PS S. University of Colorado Denver-Anschutz Medical Campus, Aurora, CO.

Body: Background: Approximately 200,000 women are diagnosed with breast cancer each year and 75% develop estrogen receptor (ER) positive tumors. Obesity is a risk factor for ER-positive breast cancer, particularly after menopause, and affects 40% of US women. Obese women are more likely to be diagnosed with advanced tumors, lymph node involvement, and less likely to respond to endocrine therapy compared to lean women. Mechanisms underlying the poorer prognosis of obese women are unclear, but epidemiological studies highlight roles for weight gain and metabolic dysfunction in breast cancer prognosis. While endocrine therapies provide considerable benefit, many obese women still experience breast cancer recurrence. Activation of growth factor receptors, including FGFR1, may contribute to endocrine therapy resistance. In this study, we describe a mouse model of obesity and breast cancer patient-derived xenograft tumors (PDX). Only the obese environment led to growth factor receptor activation, despite amplification of FGFR1. Our study highlights a role for FGF ligands, produced by adipose tissue during weight gain, in supporting FGFR1-driven tumor progression after estrogen withdrawal.

Methods: ER-positive, FGFR1-amplified breast cancer PDX tumors were established in ovariectomized lean and obese mice in the presence of estradiol (E2). To simulate the hormonal environment of postmenopausal women on aromatase inhibitors, E2 was removed from half of the mice in each adiposity group and the study was terminated 3 weeks later. Weight gain, body fat percentage, and adipose tissue as well as tumor characteristics were analyzed. A phospho-receptor tyrosine kinase array identified activated growth factor receptors in tumor lysates and results were confirmed using immunohistochemistry and the Aperio Digital Pathology system. A PDX-derived cell line was used for in vitro studies to confirm the role of ligand-activated FGFR1 in tumor cell proliferation.

Results: Prior to estrogen withdrawal (EWD), obese mice were heavier and had higher body fat percentage than lean mice. This trend was accelerated after EWD, with obese mice gaining more weight due to body fat accumulation. EWD induced excess fat deposition in visceral depots in both lean and obese mice; however, obese mice also gained fat in mammary adipose tissue. Excess mammary fat deposition in obese mice was associated with elevated production of FGF1. EWD led to tumor regression in lean, but not obese mice, measured by changes in tumor volume and Ki67 labeling. Compared to lean, tumors from obese mice had higher levels of phosphorylated FGFR1, without changes in total FGFR1.

Conclusions: Weight gain and metabolic dysfunction have been implicated in the link between obesity and breast cancer. In our study, we show that EWD promotes weight gain and mammary adipose tissue expansion in obese mice and identify a potential role for adipose-derived FGF1 in tumor progression after hormone withdrawal. Activation of growth factor receptors may underlie increased breast cancer recurrence observed in obese, postmenopausal women and could help identify patients that might benefit from FGFR-directed therapies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-06-08

Title: Characterization of an estrogen-dependent murine model of human estrogen receptor-positive breast cancer bone metastasis

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Body: Introduction: Breast cancer patients with estrogen receptor-positive (ER+) tumors have the highest rate of developing clinically evident osteolytic bone metastasis. However, much of our knowledge of breast cancer bone metastasis has been derived from ER- murine xenograft models, as robust pre-clinical models of ER+ bone metastasis are currently lacking. Thus we sought to develop and characterize a murine xenograft model of breast cancer bone metastases using an estrogen-dependent ER+ MCF-7 cell line.

Methods: 28-day-old female nude mice were implanted with the following 60-day extended-release 17β -estradiol (E_2) pellets: 0.72, 0.36, 0.18, 0.10, or 0.05 mg; with or without 1×10^5 MCF-7 cells (ATCC) inoculated via the left cardiac ventricle. Non-estradiol-supplemented female nude mice were also inoculated with either MCF-7 cells or a well-characterized, bone-tropic human ER- MDA-MB-231 cell line (referred to as MDA-SA), used as a comparison model. Radiographs and dual-energy X-ray absorptiometry's (DXAs) of hind femurs and tibias were obtained weekly to assess osteolytic lesion formation and BMD, respectively. Statistical significance was analyzed using one-way ANOVA with Tukey's post-hoc testing.

Results: Using E_2 doses previously reported in the literature to promote MCF-7 orthotopic tumors and bone metastases (0.72 mg 60-day 17β - E_2 pellets), bone metastases developed with 100% incidence ($n = 13$) by day 28 in the MCF-7 model, with osteolytic lesion area increasing to 10.3 ± 0.8 mm² by day 42 (as compared with 100% incidence on day 21 in non-estrogen-supplemented mice inoculated with MDA-SA ($n=8$) with osteolytic lesion area of 10.7 ± 2.0 mm²). However, histologic assessment of hind limbs of MCF-7 tumor bearing mice suggested that this estrogen dose stimulated a significant increase in bone mass. Therefore, the estrogen-dependency of bone metastasis formation and changes in BMD were explored. Bone metastasis incidence was estrogen-dependent; falling to less than 100% in mice with E_2 doses less than 0.72 mg (50-75%, $n = 4-12$). BMD of distal femurs of mice supplemented with estradiol was significantly greater than naïve controls on day 28 (average increase of 65.3%, $p < 0.05$), and was not dose dependent over the range of doses tested (0.05-0.72 mg pellets).

Conclusion: Despite a marked increase in bone density, ER+ MCF-7 cells formed radiographically detectable osteolytic lesions with 100% incidence by day 28 in 0.72 mg 60-day 17β - E_2 supplemented nude mice. It is possible that estrogen-stimulated increases in BMD contributed to the longer time required to achieve ER+ osteolytic lesion sizes comparable to those in standard ER- models. However, lower doses did not mitigate effects of estrogen on BMD in these 1-month-old mice, while lowering the incidence of bone metastasis formation. While further optimization is planned, intracardiac inoculation of MCF-7 cells in female nude mice provides a robust model of estrogen-dependent ER+ breast cancer bone metastases.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P4-06-09

Title: Synergistic effect of EGFR1 inhibitor and paclitaxel in newly patient derived metaplastic carcinoma cell line which harbored EGFR gene amplification

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Body: Background Metaplastic breast cancer (MBC) is a rare but aggressive subtype of invasive breast cancer, which is generally resistance to conventional chemotherapy. The majority of MBCs do not express the estrogen receptor, progesterone receptor and negative for HER-2/neu over expression. Thus, targeted treatment options to treat MBCs are also limited. Cancer cell lines are important tools used for us to test the therapeutic efficacy of anticancer agents. However, few *in vitro* systems for studying MBC are developed. Here we report a newly established novel MBC cell line using the recently developed conditionally reprogrammed cell method and search for effective therapeutic regimens to treat MBCs. **Methodology** The tumor tissue used for cell line establishment was obtained from a 40 year-old Taiwanese female, who was diagnosed with T2N2aM1 metaplastic carcinoma of breast cancer (with squamous metaplasia). The MBC tumor tissue was first digested by collagenase/ dispase mixture. After digestion, the MBC tumor cells were cultured in F medium with irradiated 3T3-J2 fibroblasts supplemented by 5 μ M ROCK inhibitor (Liu *et al.*, Am. J. Pathol. 2012). Subsequently, the MBC tumor tissue cells were passaged and propagated in these conditions until the majority of culture is tumor cells. The MBC tumor cell line was further passaged in DMEM/F12 medium without feeder cells and ROCK inhibitor. Finally, the MBC cell line (MBC-1) was characterized using DNA sequencing, immunofluorescence, Western blotting and viability assays. **Results** The genomic DNA of MBC1 was examined using DNA sequencing, and the MBC-1 maintained known patient's tumor mutation at the TP53 locus (p.V73fs*76). The MBC cell line also harbored EGFR gene amplification as the patient's tumor, according to quantitative PCR assays. To evaluate tumorigenicity, the MBC cell line was injected subcutaneously into immunocompromised mice, and the MBC tumors formed four weeks after injection. The MBC-1 stained positively using pan-cytokeratin antibody and negative of vimentin, confirming the epithelial origin of the cell line. To explore the possible effective treatment options, the cell line was incubated with different targeted therapy and chemotherapy agents, and the MBC cell line was relatively resistant to most of targeted therapy and chemotherapeutic agents. However, EGFR inhibitors (gefitinib and afatinib) could partially inhibit growth of the MBC cell line. We hypothesize that the inhibitory effect could act through inhibiting the amplified EGFR receptors. To further enhance the effect of EGFR inhibitors in the MBC-1, we combine the EGFR inhibitors with chemotherapy agents, and discovered that EGFR inhibitor and paclitaxel were synergistic across the range of concentration tested. The study on downstream mechanism of the synergism is ongoing. **Conclusions** We have successfully established a novel metaplastic carcinoma cell line with EGFR amplification from a patient using the conditionally reprogrammed cell method. Based on the preclinical study in the cell line, we found that combining EGFR inhibitor and paclitaxel could be a promising strategy to treat MBC with EGFR amplification.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-06-10

Title: Rates of successful engraftment in breast cancer xenograft models based on tissue type: Primary vs relapsed disease

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Body: Purpose: As we have published expertise in breast cancer xenograft models and clonal dynamics, our aim was to explore rates of engraftment based on type of tissue for attempted xenografting (primary vs relapsed/metastatic disease) and clinical breast biomarker subtype.

Methods: Tissue from patients (pts) enrolled in a locally advanced/metastatic study and a breast tumour tissue repository (ie. resectable primaries) between Sept. 2008 and July 2015 underwent xenografting using NodScid/IL2rgKO (NSG) mice. Xenografts were passaged when tumour volume reached 1 cm³. Mice with no engraftment after 12 months (mos) were sacrificed. Pt charts were reviewed to determine biomarker status (hormone receptor [HR], HER2), date and type of tissue collection for xenografting. Prediction of successful engraftment based on tissue type and biomarker status was performed using nominal logistic regression.

Results: A total of 70 tissue samples with known engraftment status were included in the analysis: 51 from primary breast tumour, 10 from relapsed disease (dz) with ≤ 1 line of therapy in the advanced setting and 9 from relapsed dz with > 1 line of therapy in the advanced setting. Tumours from pts treated with > 1 line of therapy were more likely to engraft compared to primary or recurrent dz with ≤ 1 line of therapy (89%, 35%, and 40% respectively; p=.008). HR- primary tumours were more likely to engraft compared to HR+ primary tumours: 71% of HR-/HER2- (triple negative) and 67% of HR-/HER2+ tumours versus 4% of HR+/HER2- and 38% of HR+/HER2+ tumours; p<.0001. Combining all tissue types, HR- tumours were more likely to engraft compared to HR+ tumours: 76% of HR-/HER2- and 67% of HR-/HER2+ tumours versus 37% of HR+/HER2+ and 22% of HR+/HER2- tumours; p=.0007. **Table 1** shows the rate of engraftment for each tissue type and biomarker status. Combining these 2 variables predicts engraftment in 80% of cases.

Conclusion: This preliminary study highlights potential differences in successful xenoengraftment based on biomarker status at diagnosis and type of tissue, primary vs relapsed tumour, the latter suggesting that the underlying biology of primary or first relapsed recurrent disease is distinct from more refractory disease, and warrants further exploration. This work is ongoing. (Funded by CBCRA, BCCF)

Engraftment of primary tumour vs relapsed disease

	Primary tumour (N=52) N, (%)	Recurrent disease and ≤ 1 line of Rx in advanced setting (N=10) N, (%)	Recurrent disease and > 1 line of Rx in advanced setting (N=9) N, (%)
Engraftment Yes	18 (35)	4 (40)	8 (89)
HR-/HER2-	10 (55)	1 (25)	2 (25)
HR-/HER2+	4 (22)	1 (25)	1 (13)
HR+/HER2+	3 (17)	0	0
HR+/HER2-	1 (6)	2 (50)	5 (62)
Engraftment No	33 (65)	6 (60)	1 (11)
HR-/HER2-	4 (12)	0	0
HR-/HER2+	2 (6)	1 (17)	0
HR+/HER2+	5 (15)	0	0
HR+/HER2-	22 (67)	5 (83)	1 (100)

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-06-11

Title: Differential regulation of microRNAs and integrins influences metastatic potential: Comparison between locally invasive BT-474 and metastatic MDA-MB-231 xenografts

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Body: Background: Generation of BT-474 and MDA-MB-231 xenografts in immunocompromised mice provides one means of dissecting the molecular events involved in local invasion versus distant metastasis. Recently, Glunde *et al* have shown an interdependence of genes involved in cell-cell and cell-matrix adhesion (E-cadherin and integrin β 1) and extra-cellular matrix degradation (MMP-2 and 9) in allowing cells to metastasize. Further, Fite *et al* have identified a set of microRNAs (miRs) up-regulated by E-cadherin (a pre-EMT marker) and down-regulated by Vimentin (post-EMT marker) in acquiring an invasive phenotype. We have performed a detailed analysis of integrins, matrix metallo-proteases and key miRs to better understand the molecular events underlying these disparate behaviours.

Methods: We injected BT-474 (N=5) and MDA-MB-231 (N=5) cells orthotopically into SCID mice. Xenografts were assessed for local growth rate and monitored for distant metastasis. The implanted tumors as well as the distant metastatic foci were harvested. Markers involved in local invasion, distant metastasis and tumor-stroma interactions including miRs were compared between BT-474 and MDA-MB-231 cell lines and their xenografts by q-RT-PCR, immunofluorescence and immunohistochemistry. **Results:** As expected BT-474 xenografts showed a higher rate of tumor growth when compared to MDA-MB-231. Histological examination of BT-474 tumors confirmed only locally invasive tumor growth with infiltrated blood capillaries and vessels; no macro and microscopic metastases were observed in the organs collected. On the contrary, MDA-MB-231 xenografts showed highly undifferentiated tumor growth and frank lung metastasis and extra-pulmonary tumor growth in one of the five mice injected despite slow rate of local growth. Expression of matrix metalloproteases – MMP-2 & 9 was more than 30 fold upregulated in MDA-MB-231 xenografts as compared to BT-474. Elevated level of E-cadherin was observed in BT-474 but was absent in MDA-MB-231.

The most interesting differences were seen in the levels of miRs and cell-surface integrins. High levels of miR-18a, miR-93 and miR-182 were observed in BT-474 implants when compared to MDA-MB-231 which had a much lower level of these miRs. On the contrary, higher levels of integrin β 3, and β 1 were observed in MDA-MB-231 tumors when compared to BT-474. Integrin β 6 was absent in both. The reciprocal relationship between these markers is being examined and compared between locally invasive tumors and metastatic triple negative breast cancers from our case series of human specimens (N=250).

Conclusion: miRs and integrins known to be involved in invasion are differentially regulated in tumors that are locally invasive compared to ones with distant metastasis. The level of the key targets of these miRs as well as additional integrins is being examined. Understanding the epigenetic regulations leading to metastasis via tumor-stroma interaction might help in discerning differential tumor behaviour.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-06-12

Title: Murine radical mastectomy model for preclinical study of adjuvant systemic therapies

Katsuta E and Takabe K. Breast Surgery, Roswell Park Cancer Institute, Buffalo, NY.

Body: Background:

Current standard of care of breast cancer is removal of primary tumor followed by systemic adjuvant therapy to reduce recurrence and to prolong survival. However, vast majority of the preclinical studies that use murine models evaluate the drug response of primary tumors either in mammary pads or subcutaneous tissues. Lately it has been shown that the genetic profiles of metastatic lung tumors are significantly different from that of their primary mammary tumors, let alone subcutaneous tumors. Therefore we hypothesized that the responses of metastatic tumors rather than primary mammary tumors need to be evaluated for a systemic therapy. However there are few reports of murine mastectomy models used for preclinical study.

Methods:

Murine mammary adenocarcinoma 4T1-luc2 cells were inoculated into #2 right mammary fat pad under direct vision as previously described (Katsuta et al, JSR 2016). The tumor burden was quantified by bioluminescence IVIS imaging system. Novel platinum drug, Triplatin, or Vehicle was administered every 4 days for 3 times from the day after inoculation. Amount of lung metastases were quantified ex vivo by IVIS imaging. Then we compared the growth of metastatic tumors between two methods of radical mastectomy; midline incision method and Halsted incision method, which were performed 8 days after inoculation. Triplatin or Vehicle was administered 2 days after mastectomies.

Results:

First we compared the two methods of chest mammary tumor removal; midline incision method and Halsted incision method. There was no significant difference in weight of resected tumors between these two techniques ($p=0.751$), however, the bioluminescence in midline incision model was significantly higher than Halsted incision model at the first day after operations ($p=0.003$). Only 1 out of 7 cases (14%) after Halsted incision method developed local recurrence, whereas all (100%) the animals that underwent midline incision method developed recurrence within 30 days after operation ($p<0.001$). No mice developed respiratory failure due to wound closure of wide skin defect. We then examined the effect of Triplatin on chest mammary tumor and lung metastasis. There was no significant difference in bioluminescence from chest mammary tumors between treatment group and non-treatment, however, ex vivo bioluminescence of lung metastases demonstrated that treatment group mice had significantly less tumor burden in lung than non-treatment group. Utilizing Halsted incision method with less local recurrence, we found that lung metastases were significantly less in treatment group than non-treatment group in live animals monitored by bioluminescence.

Conclusion:

We have established an improved murine chest mammary tumor resection model. Effects on metastases, as opposed to primary tumor should be evaluated for the preclinical study of adjuvant systemic therapy, since they may not be the same.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-07-01

Title: Profiling miRNA in high risk post-operative early breast cancer patients to detect possible association to correlation with immune system antitumor response

Tzschaschel MLJ L J, Rack B, Majunke L, Trapp EK K, Mahner S, Fasching PA A, Fehm T, Schneeweiss A, Friedl TWP WP, Polasik A, Janni W and Alunni-Fabbroni M. Ludwig Maximilians Universitaet Muenchen Klinik und Poliklinik fuer Frauenheilkunde und Geburtshilfe, Munich, Germany; University Hospital Erlangen, Friedrich-Alexander, Erlangen, Germany; Heinrich-Heine-University Duesseldorf, Duesseldorf, Germany; National Center for Tumor Diseases, University Hospital Heidelberg, Heidelberg, Germany and University Hospital Ulm, Ulm, Germany.

Body: Aims: Identifying which patient will be the one who develops metastasis or relapses is still an unsolved problem in breast cancer research. Circulating free nucleic acids such as microRNA (miRNA) are very promising cancer biomarkers to detect those patients. Aim of this study was to unravel an association among whole blood miRNA profiling, circulating tumor cells (CTCs) and clinical outcome in early breast cancer (EBC) patients and to test the utility of miRNAs as prognostic and diagnostic markers in post-operative patients.

Methods: 48 high risk EBC patients, a subsample of the German SUCCESS A trial, were included in this retrospective study together with 18 aged-matched healthy donors (HDs) as controls. Peripheral blood was drawn post-operatively before (T0), after (T1) and two years after (T2) chemotherapy directly in Tempus Blood RNA Tubes (ThermoFischer Scientific) to stabilize and isolate total RNA. miRNAs were quantified by qPCR and their relative expression was calculated according to the $2^{-\Delta\Delta Ct}$ method. CTCs were detected by immunocytochemistry. Data were analyzed using non-parametric statistical tests and p-values below 0.05 were considered significant.

Results: 8 different miRNAs, either with oncogenic (miR-10a, -19a, -21, -22) or tumor suppressive (miR-20a, -127, -155, -200b) properties, in most of the cases linked to immune system regulation (miR-19a, -20a, -21, -127 and -155) were tested. miR-19a, miR-21, miR-22 and miR-127 showed the most promising results as prognostic markers in our tests. They could differentiate patients from HDs (Patients vs. HD: miR-19a at T0, $p=0.004$), T1 ($p<0.0001$), miR-21 at T0 ($p=0.001$), T1 ($p=0.004$), miR-22 at T1 ($p=0.012$), T2 ($p=0.034$)). Additionally they correlated with the post-operative detection of CTCs (miR-127) and the late development of metastasis (miR-19a). Kaplan-Meier analysis indicated a borderline association between a longer progression free survival and higher miR-19a levels (HR= 2.808, 95% confidence interval [CI] =0.904, $p=0.074$.)

Conclusion: We found a panel of miRNAs that could discriminate post-operative patients from HDs and could predict patients' outcome. We suggest that whole blood miRNA profiling could unravel a correlation with the activation of the immune system, leading to an efficient antitumor response and a better clinical outcome. These findings could open new perspectives in immune oncology and personalized therapy already in the early phase of the disease.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-07-02

Title: Expression of miR-18a and miR-210 in normal breast tissue as candidate markers of breast cancer risk

Wang J, Shidfar A, Costa FF F, Scholtens D, Bischof JM M, Sullivan ME E, Ivancic D, Soares MB B and Khan SA A. Feinberg School of Medicine, Northwestern University, Chicago, IL.

Body: Purpose: miRNAs are non-coding RNAs that are abnormally expressed in breast cancer, with critical roles in cancer due to their regulation of large gene networks. miRNA expression in benign high-risk breast tissue has never been evaluated, but it may provide information about early dysregulation events that contribute to breast cancer risk. The contralateral unaffected breast (CUB) of women with unilateral breast cancer is in high-risk for the second primary cancer. Thus, we examined miRNA expression profiles in tumor and the matching CUBs to seek potential miRNA biomarkers for breast cancer risk.

Methods: FFPE tissues of breast cancer and their matching CUB tissues were sectioned. The areas of tumor and normal ductal epithelium were outlined and then dissected using laser microdissection system. Total RNA was extracted for miRNA profiling studies. Expression profiles of 754 mature miRNAs were examined using TaqMan Low Density Arrays assays in 30 paired breast cancer and CUB samples (15 with ER+ tumors, 15 with ER- tumors) and 15 reduction mammoplasty (RM) controls, matched by age, race and menopausal status. ANOVA test was performed to examine the differential expression among groups and pairwise comparison with Sidak adjustment was used for multiple comparison. Seven candidate miRNAs were then examined in an independent CUB sample set (20 with ER+ tumors, 20 with ER- tumors) and 20 RM controls. Further independent validation was performed using qRT-PCR in 80 benign breast biopsy (BBB) samples: 40 from women who subsequently developed breast cancer (cases) and 40 from those who did not (controls). Logistic regression analysis and receiver operating characteristic (ROC) analysis were performed using combinations of the expression of multiple miRNAs to establish models discriminating cases from controls.

Results: Seven miRNAs (miR-18a, miR-210, miR-214*, miR-124, miR-193a-3p, miR485-3p and miR-671-3p) were found to be differentially expressed in breast cancer and CUB samples vs. RM samples in the discovery sample set. Among them, miR-18a and miR-210 were validated in a second, independent CUB sample set. The expression of miR-18a and miR-210 were significantly higher in tumor (regardless ER status) compared to CUBs and RM controls. The expression levels in CUBs were significantly higher than in RM. We then examined miR expression in case BBB samples, and confirmed that expression of miR-18a and miR-210 were increased compared with controls. ROC analysis using miR-18a and miR-210 discriminated high-risk cases from standard-risk controls with OR 2.44, P = 0.022.

Conclusion: The expression of miR-18a and miR-210 were elevated in breast cancer, in matched CUBs, and in BBB predating cancer diagnosis. These data provide strong support for the hypothesis that miR-18a and miR-210 expression in BBB is an indicator of increased risk of breast cancer. Given the high expression in tumors, they are also potential cancer detection biomarkers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-07-03

Title: Dynamic regulation of a microRNA-mRNA network during breast cancer metastasis reveals an essential tumor-promoting role for miR-203

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Body: BACKGROUND: Metastasis represents a critical turning point from curable to incurable breast cancer. Alterations in gene expression underlie aspects of this transition and are coordinated both genetically and epigenetically.

HYPOTHESIS: Epigenetically controlled microRNA-mRNA regulatory networks underlie the ability of breast cancer cells to metastasize and acquire treatment resistance.

METHODS: Human MDA-MB-231 and MDA-MB-436 breast cancer xenograft primary tumors and corresponding lymph node, liver, lung and diaphragm metastases were generated by inoculation of cell lines into the mammary fat pad of NSG mice. Human-specific gene expression profiling was performed using microarrays with qPCR validation. MicroRNA targets were validated by 3'UTR mutagenesis-luciferase assays. Lentiviral shRNA and miRNA mimic constructs were stably transduced into the same cell lines and growth properties were assessed *in vitro* and *in vivo*. Mice were euthanized when tumors achieved 10% of body weight or at day 100, whichever came first.

RESULTS: A common set of 18 mRNAs was differentially regulated at all 4 metastatic sites relative to primary tumors. Of these, 17 were downregulated, and 13 of these were strikingly enriched for microRNA binding sites. In the same tissues, we identified 21 microRNAs that underwent downregulation during primary tumor growth relative to parental cell cultures, but were highly upregulated at all 4 metastatic sites. 18 of these had at least one target among the downregulated genes, and 6 had multiple targets in the same 13 mRNA 3' UTRs. One, miR-203, previously described as a tumor suppressor, directly targeted two of the repressed genes, TWF1 and APBB2. MiR-203 levels positively correlated with primary tumor size in METABRIC ($p=0.0096$). MiR-203 overexpression blunted *in vitro* tumorigenic properties, modestly reduced primary tumor growth rates, and prevented metastasis formation. Remarkably, knockdown of miR-203 also reversed multiple tumorigenic properties of both cell lines, including proliferation, migration, and growth in soft agar, and conferred a fibroblastic morphology on MDA-MB-231 cells. In addition, primary miR-203KD xenograft tumors grew extremely slowly and underwent partial involution between days 80-100. Residual miR-203KD tumors and metastases at day 100 demonstrated re-gain of miR-203 expression through loss of the shRNA silencing vector.

CONCLUSIONS: A small group of microRNAs displays biphasic expression during cancer growth. At least one of these, microRNA-203, is required for metastatic growth, but also inhibits metastasis formation when expressed at high levels. Our findings suggest opposing roles for the same microRNA at different stages of breast cancer progression, and support the existence of dynamic, context-sensitive epigenetic mechanisms that adapt breast cancer cells to thrive at remote sites. These mechanisms may serve as targets for intervention during the evolution of metastatic disease.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-07-04

Title: Detecting early breast cancer by the combination of five serum microRNAs and its possibility of prediction of pathological complete response in neoadjuvant chemotherapy

Shimomura A, Shiino S, Kawauchi J, Takizawa S, Sakamoto H, Shimizu C, Takeshita F, Niida S, Kinoshita T, Tamura K and Ochiya T. National Cancer Center Hospital, Tokyo, Japan; Toray Industries, Inc., Kamakura, Japan; National Cancer Center Research Institute, Tokyo, Japan and National Center for Geriatrics and Gerontology, Nagoya, Japan.

Body: [Background] It is recently reported that microRNAs (miRNAs) are stably present in serum and potentially useful in the diagnosis and evaluation of treatment of cancer.

[Materials and Methods] Serum samples of breast cancer before treatment (n=1280) between 2008 and 2014 were obtained from National Cancer Center Hospital and controls (n=3348) were obtained from collaborative institutes. Additionally, the serum sample of patients who received neoadjuvant chemotherapy (NAC) and surgery between last chemotherapy administration and surgery were collected. A comprehensive quantitative expression analysis of miRNA was performed using the by DNA chip "3D-Gene® (Toray Industries Inc.)" Clinicopathological data was retrieved from medical records. Pathological complete response (pCR) was defined as the absence of residual invasive and in situ cancer of the resected breast specimen and all sampled regional lymph nodes.

[Results] Serum samples before treatment of breast cancer patients (n=74), non-cancer controls and patients with other cancers were used (n=2007) in a training set. The rest except for samples after NAC were used in a test set. The formula with the combination of five miRNAs (miR-1246, miR-1307-3p, miR-4634, miR-6861-5p, and miR-6875-5p) was found to be able to detect breast cancer (BCmiR set). BCmiR set had a sensitivity of 97.3%, specificity of 82.9% and accuracy of 89.7% in the test cohort. BCmiR set could detect breast cancer in the non-invasive stage (sensitivity of 98.0% for Tis).

In the breast cancer patients, 91 patients received NAC and surgery. Median age of NAC patients was 49 years (range 28-77). Forty-two patients were hormone receptor-positive (HR+) and HER2-negative (HER2-), 24 were HR+ and HER2-positive (HER2+), 11 were hormone receptor-negative (HR-) and HER2+ and 14 were HR- and HER2-. pCR was observed in 19 (20.9%) of NAC patients. pCR in each subtypes were 3 (7.7%) in HR+ and HER2-, 6 (33.3%) in HR+ and HER2+, 4 (57.1%) in HR- and HER2+ and 3 (27.3%) in HR- and HER2-. Serum after NAC were obtained from 19 pCR patients and 71 non-pCR patients. When we applied BCmiR set to the serum samples after NAC, the average diagnostic index (cut-off value=0) in pCR patients was significantly lower than that in non-pCR patients (pCR, -0.30 ± 0.84 ; non-pCR, 0.31 ± 1.15 ; $p=0.03$). In fact, 57.9% of pCR patients were classified into non-breast cancer. However, 40.8% of non-pCR patients were misclassified into non-breast cancer.

[Conclusion] The combination of five miRNAs (BCmiR set) measured from serum can be used to detect breast cancer. BCmiR set has potential to predict pCR in patients who received NAC. The further analysis to predict pCR is underway and further results will be presented in the symposium.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-07-05

Title: MicroRNA-27b mediates deregulation of energy metabolism in breast cancer

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Body: MicroRNAs (miRNAs) are a class of small, non-coding RNA which participate in gene regulation by targeted silencing of specific mRNA transcripts. Recently, it has become clear that dysfunctional miRNA expression is an important contributor to the deregulation of key signaling pathways underlying carcinogenesis. MicroRNA-27b is an example of one such pro-oncogenic miRNA, and correspondingly, its expression has been found to be upregulated in breast cancer cells and tissues. In this regard, several targets of miR-27b have previously been verified, and implicate miR-27b in multiple neoplastic processes including migration, invasion and metastasis. In order to enable future development of miRNA-based cancer therapies, the putative targets of each miRNA must be mapped more comprehensively. Such awareness is necessary in order to make informed choices regarding whether a miRNA-based therapy is likely to be effective and what side-effects should be anticipated. Therefore, this project seeks to further establish the role of miR-27b in the context of breast cancer, specifically by identifying novel target transcripts under its control. Using target prediction algorithms and breast cancer cell culture models, initial evidence has implicated Pyruvate Dehydrogenase Protein X (PDHX) to be a probable target. As a structural component of the Pyruvate Dehydrogenase Complex, the role of PDHX in linking the end products of glycolytic reactions with subsequent oxidative phosphorylation pathways is indispensable. However, a hallmark of cancer cell metabolism is the propensity to consume glucose aerobically, which liberates additional carbon skeletons for use in biosynthetic reactions that are crucial for tumor growth. In this way, miR-27b overexpression appears to sever the link between glycolysis and subsequent catabolic pathways, thus conferring a pro-growth advantage to the cancer cells. Expression profiling of PDHX using both breast cancer cell lines and human breast tissues (which overexpress miR-27b) reveal that PDHX levels are indeed significantly reduced, thus supporting the notion that miR-27b alters cancer cell metabolism specifically by targeting this gene in breast cancer. Improving the current understanding of miR-27b's functions will lay the necessary ground work to evaluate its potential as a novel target for cancer therapy. Similarly, the awareness of the expression status of miR-27b and its targets in breast cancer tumors will further efforts to find suitable miRNA tissue biomarkers to enhance screening and prognosis. In this way, microRNA-based approaches offer the distinct potential to improve cancer detection as well as treatment, making them a dynamic method to improve management of breast cancer patients in the future.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-07-06

Title: MicroRNAs associated with acquired taxane resistance in a breast cancer cell line model

Taylor KJ J, Chong T, D'Costa A, Yao C, Gourley C, Cameron DA A, Bartlett JMS MS and Spears M. Edinburgh Cancer Research Centre, University of Edinburgh, Edinburgh, United Kingdom; Ontario Institute of Cancer Research, Toronto, ON, Canada and University of Toronto, Toronto, ON, Canada.

Body: Background: Acquired chemoresistance remains the major cause of therapeutic failure in the treatment of breast cancer. Improved knowledge of the transition from drug sensitive to resistant breast cancer will pave the way for novel preventative and therapeutic strategies. MicroRNAs (miRNA) are endogenous, small non-coding RNAs that regulate gene expression by targeting the 3'UTR region of messenger RNAs. There is a growing body of evidence to suggest miRNAs may be involved in the development of chemoresistance and may play a role in the regulation of drug resistance pathways.

Methods: An *in vitro* model of paclitaxel resistance was developed through the generation of resistant MDA-MB-231 cell lines by serial culture in escalating doses of taxane until resistance was achieved. The chemoresistant model was used to compare differential miRNA expression with the sensitive, parental line using the Nanostring® platform, analysing 800 human miRNAs. Confirmation of differential expression was performed by QRT-PCR.

Results: This analysis resulted in 30 significantly altered miRNA (1.5 fold, p value < 0.05) at 25nM paclitaxel and 48 significantly altered miRNA at 50nM paclitaxel. The top up-regulated miRNA cluster in MDA-MB-231 25PACR is hsa-miR-548l (fold change: 2.89, p value: 0.016) and top down-regulated was hsa-miR-449a (fold change: -4.1, p value: 0.001). In MDA-MB-231 50PACR the top up-regulated miRNA cluster is hsa-miR-193a-5p (fold change: 3.746, p value: 0.008) and the top down-regulated miRNA cluster is hsa-miR-135a (fold change: -4.085, p value: 0.001). To explore the molecular mechanisms of the differentially expressed miRNAs in paclitaxel resistance, targets were predicted by *in silico* analysis. Pathways and networks designated by miRNA targets included the cell cycle, PI3K/Akt pathways and focal adhesion.

Conclusion: In this study we identified candidate resistance-associated miRNAs which were differentially expressed between *in vitro* derived paclitaxel resistant MDA-MB-231 and the sensitive parental line. Further validation to ascertain their role in the transition to a chemoresistant phenotype is currently ongoing.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-07-07

Title: Reduced expression of miR-200c-3p contributes to cell migration and invasion in paclitaxel-resistant breast cancer cell

Wang X, Chen J, Chen Z, Huang J and Ye W. Zhejiang Cancer Hospital, Hangzhou, Zhejiang, China.

Body: Background: miRNAs are a class of small noncoding RNA molecules that regulate gene expression by degradation of target mRNAs or inhibition of translation. Metastasis is one of the major problems that give obstacles to improve the clinical outcome of breast cancer. However, the underlying molecular mechanism of breast cancer metastasis remains largely unclear. Emerging studies have demonstrated that miRNAs play an important part in tumor invasion and metastasis. In the present study, we aimed to investigate the role and mechanism of miR-200c-3p in regulating the migration and invasion of paclitaxel-resistant breast cancer cells, and identify potential targets for breast cancer treatments.

Material and methods: Paclitaxel-resistant breast cancer cell line MCF-7/Tax was established by stepwise selection in increasing concentration of paclitaxel. The 50% inhibitory concentration and cell viability were measured by the MTT assay. The expression level of miR-200c-3p in MCF-7/Tax and the parental MCF-7 cells was assessed by quantitative real-time RT-PCR. Gain-of-function and loss-of-function study on cell migration and invasion abilities were carried out by transfection of miR-200c-3p mimics or inhibitors respectively. The molecular target of miR-200c-3p was verified by dual-luciferase reporter assay.

Results: Paclitaxel-resistant breast cancer cell line MCF-7/Tax was established. Compared with parental MCF-7 cells, expression of miR-200c-3p determined by real-time RT-PCR was significantly down-regulated in paclitaxel-resistant MCF-7/Tax cells. The ability of migration and invasion of paclitaxel-resistant MCF-7/Tax cells was obviously increased as compared to parental MCF-7 cells. In addition, we found that up-regulation of miR-200c-3p expression inhibited the migration and invasion of MCF-7/Tax cells, while down-regulation of miR-200c-3p expression increased the migration and invasion of MCF-7/Tax cells. Dual-luciferase reporter assay demonstrated that SOX2 was one of the direct targets of miR-200c-3p in breast cancer.

Conclusions: Our results indicate that reduced expression of miR-200c-3p plays a crucial role in cell migration and invasion of paclitaxel-resistant breast cancer cell, and miR-200c-3p may suppress the cell migration and invasion possibly partially through SOX2. Our findings suggest that miR-200c-3p may serve as a promising therapeutic target for breast cancer metastasis.

Keywords miRNA · Breast cancer · Invasion

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Publication Number: P4-07-08

Title: Age-related microRNAs and their biomarker potential in chemotherapy-treated older breast cancer patients

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Body: BD and SH contributed equally to this study.

Background

MicroRNAs (miRNAs) are important regulators of cellular function and have been associated with both aging and cancer, while the impact of chemotherapy on miRNAs has barely been studied.

Patients and Methods

To examine whether chemotherapy accelerates the aging process, we have monitored age-related circulating miRNAs in 89 older breast cancer patients (≥ 70 y), receiving adjuvant chemotherapy (N= 46; chemo group, ChG) or no chemotherapy (N= 43; control group, CoG). Patients and associated blood samples belonged to the cohort of our recently published study (Brouwers et al., Oncotarget. 2016.) All patients underwent geriatric assessment at inclusion (T0), after 3 months (T1) and 1 year (T2). At each timepoint we analysed the serum expression of nine age-related miRNAs (miR-20a, miR-30b, miR-34a, miR-106b, miR-191, miR-301a, miR320b, miR374a, miR-378a).

Our primary aim was to assess miRNA changes during the study period, including differences between groups. Secondary endpoints included association of microRNAs with: chronological age, clinical geriatric assessment parameters and aging biomarkers assessed in the above-mentioned study. We then investigated the predictive role of miRNAs at T0 on: decline in functionality and quality of life, toxicity and unexpected hospitalization during or after chemotherapy. We also performed clustering of patients according to specific miRNA signatures.

Results

Except for miR-106b, which appeared to behave slightly different in ChG compared to CoG, all other miRNAs underwent moderate fluctuations during the study course with no significant differences between both groups. Also within the older cohort, several age-related miRNAs significantly ($p < 0.05$) correlated with clinical aging/frailty (miR-106b, miR-191, miR-301a, miR-320b, miR-374a), as well as with other biomarkers of aging. In particular, miR-106b, miR-374a and miR-378a were associated with IL-6 (slope= -0.34, -0.30 and 0.30 respectively, $p < 0.05$), whereas miR-301a and miR-378a showed a relevant correlation with MCP-1 (slope= -0.18 and 0.27 respectively, $p < 0.05$).

Moreover, based on their 'aging miRNA' profiles, patients clustered into two distinct groups, cluster A (CA) and cluster B (CB), exhibiting significantly different results for several biological/clinical aging parameters. CA (N=43, miR-20a, miR-30b, miR-191, miR-301a and miR-374a underexpressed, miR-378a overexpressed) was characterized by older age, higher geriatric risk profile, as well as elevated IL-6, TNF α and MCP-1 levels compared to CB (N=45, inverse expression pattern). Moreover, 31.9% of CA patients but only 7.4% of CB patients experienced decline in quality of life after chemotherapy ($p = 0.051$).

Conclusions

These results further corroborate our recent findings, stating that adjuvant chemotherapy does not significantly boost aging progression in elderly breast cancer patients. Our data also endorsed specific age-related miRNAs as promising aging/frailty biomarkers in oncogeriatric populations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-07-09

Title: Differential expression of microRNAs (miRNAs) in HER2 negative breast cancer (BC)

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Body: Background:

MiRNAs are non-coding single-stranded RNAs that control gene expression by directing their target mRNAs for degradation and/or posttranscriptional repression. Compared to mRNA signatures, miRNAs have better and stronger biomarker properties with 20 times more power in biomarker studies as compared to mRNAs (when comparing 20,000 mRNAs to ~ 1,000 miRNAs). Global downregulation of miRNAs has emerged as a common theme in human breast tumors and has been shown to contribute to oncogenesis

Hormone receptor (HR) status is one of the most important factors influencing BC outcome. Several data indicate the extensive alterations in miRNAs regulation upon estrogen pathway and suggest the utility of considering miRNAs expression in the understanding the development of cancer disease, invasiveness, metastasis and treatment failure.

Our aim was to identify differential expression miRNAs in HER2 negative BC. We previously reported a set of miRNAs deregulated in HR+/HER2- BC vs. Triple negative BC (TNBC). In this work we have validate these differences in a new cohort of BC patients.

Methods:

Total RNA was extracted samples extracted from primary breast cancer FFPE tissue. MicroRNAs expression was analyzed by RT-qPCR using TaqMan Arrays from Applied Biosystems. Normalization was performed using de DCt method with two housekeeping miRNAs identified previously identified using NorMean. MicroRNA expression values in each group were compared by the Mann Whitney test.

Results:

121 patients with early HER2 negative BC were included in the present study. All patients showed absence of nodal involvement at the time of diagnosis. The median age at diagnosis was 56 years, 89 cases (74%) had positive estrogen receptor and 53 cases (44%) grade 3. miR-20a, miR-19a, miR-106a, miR-18a and miR-135b expression were significantly higher in TNBC samples, whereas miR-190b, miR-375, miR-449a, miR-342, miR-149, miR-193b, miR-214, miR-30a* and miR-30e* were significantly more expressed in ER+/HER2- samples. No significant differences were found in miR-139-5p expression between both groups.

Conclusions:

We have validated fourteen miRNAs that are differentially expressed between ER+/HER2- and TNBC breast cancer subtypes. Altered expression of miRNAs could be a potential therapeutic tool and promising biomarker in personalized treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-07-10

Title: Epithelial mesenchymal transition associated with high miR-221 and integrin β 6 leads to poor prognosis in hormone receptor positive HER2 negative breast cancers

Prabhu JS S, Kaul R, Korlimarla A, Desai K, Gangadharan C, Rajarajan S, Nair MG G, Alexander A, Kaluve R, Manjunath S, Correa M, Prasad MSN, Patil S, Srinath BS and Sridhar TS. St Johns Research Institute, Bangalore, Karnataka, India; St Johns Medical College Hospital, Bangalore, Karnataka, India; St Johns Medical College, Bangalore, Karnataka, India and Shankara Cancer Hospital and Research Center, Bangalore, India.

Body: Background: MicroRNA mediated molecular alterations are involved in the initiation and progression of cancer. Altered expression of multiple microRNAs is associated with endocrine resistance in hormone receptor positive HER2 negative (HR+/HER2-ve) cancer. The role of miR-221 in inducing epithelial to mesenchymal transition (EMT) is well documented especially in cell line model systems. However, the detailed mechanism of specific microRNAs in intrinsic and acquired resistance to endocrine therapy needs to be worked out. In addition, more needs to be done in the documentation of these mechanisms in human breast cancer specimens with complete clinical documentation and long-term follow-up. In this study, we have evaluated the clinical significance of miR-221 and its mechanistic role in EMT using human specimens and cell line models.

Materials and Methods: Formalin fixed paraffin embedded tumor from 129 HR+/HER2-ve breast cancer patients with a median follow up of 63 months were used for estimation of miR-221 by quantitative real time PCR. Expression levels of genes which are direct targets of miR-221 and related genes in EMT were analysed from these tumors. Survival between miR-221 high and low groups was compared by Kaplan Meier survival curves and prognostic relevance was estimated by Cox proportional hazard model.

Cell line experiments to investigate the role of miR-221 in inducing EMT through integrin β 6 are underway in both wild type and tamoxifen resistant MCF-7 cell lines (A gift from Prof Ben Ho Park, Johns Hopkins University School of Medicine).

Results: A significant elevated level of miR-221 was observed in small proportion (14%) of HR+/HER2-ve tumors. miR-221 expression had an inverse correlation with both ER protein and ESR1 mRNA levels within HR+/HER2-ve tumors. Tumors with high levels of miR-221 showed significantly higher expression of integrin β 6 which is a robust marker of EMT. Patients with high expression of miR-221 had a poorer survival in Kaplan Meier analysis.

Results of interrogation of EMT mediated through integrin related pathways involving miR-221 in cell line models will be presented.

Discussion: The association between miR-221 and integrin β 6 in HR+/HER2-ve breast cancer with endocrine resistance suggests a potential link between an epigenetic regulator and a mediator of tumor-stromal interaction. The other mediators involved in this pathway are being investigated. miR-221 could be potentially used as a marker for identification of a poor prognostic subtype within HR+/HER2-ve breast cancers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-07-11

Title: MicroRNA-367a promotes tumor growth and stemness potential by targeting FBXW7 in breast cancer

Xiao G, Zhang J, Sun X, Qin S, Zhang B, Liu D, Liu J and Ren H. The First Affiliated Hospital of Medical College, Xi'an Jiaotong University, Xi'an, Shaanxi, China.

Body: Increasing studies showed that MicroRNAs (miRNAs) participate in the carcinogenesis and progression of breast cancer, but the specific mechanism of miRNAs in breast cancer needs to be further explored. In this study, we found that miR-367a was significantly up-regulated in breast cancer tissue samples and cell lines, compared to paired normal tumor-adjacent samples and MCF-10A. Meanwhile, Kaplan–Meier analysis demonstrated high miR-367a expression was evidently correlated with poor prognostic features of breast cancer. Overexpression of miR-367a remarkably promoted proliferation, cell cycle, inhibited apoptosis ratios and stem-like capacity of breast cancer cells in vitro, while silencing the expression of miR-367a dramatically reversed these cellular events. In addition, in vivo studies showed that knockdown of miR-367a inhibited tumor growth of breast cancer. Moreover, F-box and WD repeat domain-containing 7 (FBXW7) was identified as a direct target gene of miR-367a, as miR-367a directly bound to the 3' untranslated region of FBXW7. Notably, alterations of FBXW7 levels abrogated the effects of miR-367a on breast cancer cell proliferation, cell cycle, apoptosis and stemness potential. In conclusion, evidence from this study demonstrated that miR-367a may serve as a novel prognostic indicator for breast cancer patients and exerts tumor promotion, at least in part, by inhibiting FBXW7.

Key words: miR-367a, FBXW7, breast cancer, tumor growth, stem-like capacity.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-08-01

Title: IsomiRs and tRNA fragments are race-dependent regulators in breast cancer

Rigoutsos I, Telonis AG G, Loher P, Jing Y and Londin E. Computational Medicine Center, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA.

Body: For a long time, it was believed that each microRNA (miRNA) precursor arm produces one consequential mature miRNA (the “archetype” miRNA). This product is the sequence that one finds listed in databases such as miRBase. We now know that each arm produces clouds of isoforms (isomiRs) that include the archetype and contribute to the functional role of the miRNA locus from which they arise.

By analyzing the transcriptomes of healthy individuals from the 1000 Genomes Project (1KG) we showed that isomiR expression is constitutive and depends on a person's sex, population origin, and race. Using the breast cancer (BRCA) datasets from The Cancer Genome Atlas (TCGA) repository we extended our findings to the disease context and showed that isomiR expression also depends on a patient's race and on BRCA subtype.

We have tested several isomiRs from two different oncogenic miRNA loci. In particular, we selected isomiRs that are differentially abundant between White (Wh) and Black or African American (B/Aa) Triple Negative Breast Cancer (TNBC) patients. Transient transfection of each isomiR in MDA-MB-468, a cell line model of TNBC in B/Aa patients, and BT-20, a cell line model of TNBC in Wh patients, led us to two observations: 1) within a cell line, isomiRs from the same locus downregulated largely distinct collections of mRNAs; and, 2) across cell lines, isomiRs from the same locus downregulated largely distinct collections of mRNAs. *The findings suggest that distinct isomiRs from the same miRNA locus target different mRNAs in Wh and in B/Aa TNBC patients.*

With regard to tRNAs, the conventional understanding was that the genomic loci encoding them produce a precursor transcript which is processed to give rise to the mature tRNA used in codon translation. Early efforts showed that, in addition to the full-length mature tRNAs, tRNA fragments (tRFs) are also produced from both the precursor and the mature tRNAs. These tRFs were initially thought to be degradation products.

Our analyses of transcriptomes from the 1KG showed that tRFs in healthy individuals are constitutive and their profiles depend on race, sex, and population origin. Our findings extended to the disease context: using TCGA BRCA transcriptomes, we showed that tRF expression also depends on a patient's race and on BRCA subtype. We also discovered a new category of tRFs, the “internal tRFs” or i-tRFs that are wholly internal to the mature tRNA. *Our analyses show that i-tRFs are responsible for much of the difference we observe across individuals.*

We examined experimentally several tRFs with abundances that differ between Wh and B/Aa TNBC patients. Transient over-expression of these tRFs in the MDA-MB-468 and BT-20 cell lines led to findings analogous to those we made for isomiRs: 1) within each cell line, each tRF downregulated largely different groups of mRNAs; and, 2) the same tRF downregulated largely different groups of mRNAs in the two cell lines. *The findings suggest that distinct tRFs can have distinct mRNA targets that differ between Wh and B/Aa TNBC patients.*

These findings suggest that isomiRs and tRFs are regulatory molecules with previously unrecognized roles that depend on a patient's race.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-08-02

Title: Integration of transcriptomic, proteomic and drug response data in triple negative breast cancer cell lines and PDX models

Mills CE E, Hafner MA A and Sorger PK K. Harvard Medical School, Boston, MA.

Body: Drug response screens on panels of cell lines aimed at identifying markers of sensitivity or resistance have been limited in their successes. Unfortunately, the recent release of many such studies has been accompanied by concerns surrounding reproducibility. Since then, several publications have addressed these concerns by pointing out sources of variability and by suggesting better experimental methods as well as more robust analytical approaches. In the presented profiling effort, we integrated the latest advances in drug response measurement and focused on data diversity and quality rather than on breadth. We selected 32 breast cancer cell lines with a strong bias towards triple negative lines as well as 4 cell lines established from relevant patient-derived xenografts. We used high content microscopy to assay the phenotypic responses of the cell lines to a panel of 34 drugs made up largely of kinase inhibitors currently in the clinic along with some standard of care chemotherapeutics. The microscopy based drug response assay allowed us to measure drug potency, and to quantify the efficacy of the drugs in terms of growth inhibition and cell death. For the same cell lines, we used RNAseq to measure basal mRNA expression levels and shotgun mass spectrometry to measure endogenous protein levels.

The completeness and controlled conditions under which these data sets were collected provide confidence in their integration. The complementarity of these multi-omics data has allowed us to address questions about the landscape of, particularly triple negative, breast cancer cell lines. Such questions include: where do the patient-derived lines lay among the established cell lines? and how different are the landscapes defined by drug response phenotypes, mRNA expression, and protein levels? We used network-based algorithms to identify eigenstates of signaling pathways related to genomic events, and further explored these states in the TCGA data.

At the level of drug response, we have focused on important questions related to the clinical use of kinase inhibitors. In particular, we compared various CDK inhibitors in an effort to identify markers that are informative of response potency and efficacy. We have also looked at variability of the responses of the cell lines studied to multiple PI3K inhibitors that either target specific isoforms or all isoforms. Overall the data set that has been collected is a valuable resource for understanding drug response in triple negative breast cancer, and the transcriptomic and proteomic factors that influence it.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-08-03

Title: Mathematical modeling of the unfolded protein response pathway in breast cancer cells

Shajahan-Haq AN N, Demas DM M, Clarke R and Baumann WT T. Georgetown University Medical Center, Washington, WA and Virginia Tech, Blacksburg, VA.

Body: Over 70% of all breast cancers are estrogen receptor positive (ER+) and are treated with endocrine therapies such as aromatase inhibitors or ER-disruptors as part of the standard of care. However, endocrine resistance remains a major clinical challenge for the successful treatment of this disease. ER+ breast cancer cells can escape endocrine therapy-mediated cell death through adaptations in the unfolded protein response (UPR), an evolutionarily conserved cellular stress response pathway that is linked to many other pathways (autophagy, metabolism, anti-oxidant response). To better understand how the UPR pathway promotes endocrine resistance, we built a mathematical model from quantitative measurements of key UPR signaling molecules following treatment of ER+ MCF7 cells (endocrine sensitive, estrogen dependent) with dithiothreitol (DTT). DTT disrupts or prevents protein disulfide bonding and is a potent inducer of the UPR. The same measurements were also made in two MCF7-derived resistant cell lines: LCC1 (sensitive to ER-disruptors, estrogen independent) and LCC9 (resistant to ER-disruptors, estrogen independent). Determining the (minimal) changes to the MCF7 mathematical model needed to adequately capture the data from the resistant cell lines allows us to create specific hypotheses for how the UPR adapts to help produce resistance to endocrine therapies. These hypotheses, in turn, can lead to drug targets for reversing resistance.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-09-01

Title: Tumor suppressor control of the cancer stem cell angiogenic niche

Crowe D, Kramer K and Wu J. University of Illinois, Chicago, IL.

Body: Mammary stem cells (MSC) expansion is associated with aggressive human breast cancer. The nuclear receptor peroxisome proliferator activated receptor γ (PPAR γ) is a breast cancer tumor suppressor, but the mechanisms of this suppression are not completely characterized. To determine if PPAR γ regulates MSC expansion in mammary cancer, we deleted PPAR γ expression in the mammary epithelium of an in vivo model of basal breast cancer. Loss of PPAR γ expression reduced tumor latency, and expanded the CD24⁺/CD49^{fhi} MSC population. PPAR γ null mammary tumors exhibited increased angiogenesis which was detected in human breast cancer. In vivo inhibition of a PPAR γ regulated miR-15a/angiopoietin-1 pathway blocked increased angiogenesis and MSC expansion. PPAR γ bound and activated a canonical response element in the miR-15a gene. PPAR γ null tumors were sensitive to the targeted anti-angiogenic drug sunitinib but resistant to cytotoxic chemotherapy. Normalization of tumor vasculature with sunitinib resulted in objective response to cytotoxic chemotherapy. Chemotherapy treated PPAR γ null mammary tumors exhibited luminal phenotype and expansion of unipotent CD61⁺ luminal progenitor cells. Transplantation of chemotherapy treated luminal progenitor cells recapitulated the luminal phenotype. These results have important implications for anti-angiogenic therapy in breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-10-01

Title: Estrogen induced apoptosis can be mimicked by targeting unfolded protein response

Sengupta S, Sevigny C and Clarke R. Georgetown-Lombardi Cancer Center, Georgetown University, Washington, DC.

Body: Estrogen signaling is considered to promote growth in estrogen receptor positive (ER+) breast cancers and its actions can be blocked by antagonists like tamoxifen or by inhibiting the synthesis of estrogen by aromatase inhibitors. Both of these classes of drug are used to treat ER+ breast cancers in the clinic. Paradoxically, before the discovery of tamoxifen and aromatase inhibitors high dose estrogen (HDE) was the choice of endocrine therapy to treat post-menopausal breast cancers. Recent clinical trials have observed 30% clinical benefit rate with high as well as low doses of estrogen-therapy in aromatase-inhibitor resistant breast cancers. Despite its clinical success, the precise underlying mechanism of estrogen-therapy by which it triggers the tumor regression remains unknown. Studies in the laboratory have indicated that unfolded protein response (UPR) and apoptotic pathways may play important role in estrogen-induced apoptosis. Using MCF7:5C cells, which can proliferate independent of estrogen and are hyper-sensitive to estrogen as evident by induction of apoptosis, we demonstrate that increased global protein translational load as the trigger for estrogen-induced apoptosis. This subsequently leads to endoplasmic reticulum (EnR) stress and activates the protein kinase-RNA-like endoplasmic reticulum kinase (PERK) pathway of UPR. Our results also suggest that sustained phosphorylation of eukaryotic initiation factor 2-alpha (eIF2- α), a downstream target of PERK activation, may be crucial in estrogen-induced apoptosis. Phosphorylation of eIF2- α attenuated global translation but preferentially allowed high expression of transcription factors, including, activating transcription factor 4 (ATF4) and C/EBP homologous protein (CHOP). ATF4 and CHOP are known to activate apoptosis. Notably, we were able to recapitulate this phenotype by pharmacologically inhibiting the regulatory subunits of the protein phosphatase 1, GADD34 (growth arrest and DNA damage inducible protein) and CReP (constitutive repressor of eIF2 α phosphorylation), that are responsible for de-phosphorylation of eIF2- α . This was evident in MCF7:5C cells as well as another estrogen-independent breast cancer cell line LCC9 that is resistant to both tamoxifen and fulvestrant but does not undergo estrogen mediated apoptosis. We further observed that the combination of 4-hydroxy-tamoxifen (4OHT) and pharmacological inhibitors of GADD34 and CReP potentiated its apoptotic action in both LCC9 and MCF7:5C cells. These results not only enhance our understanding of the apoptotic mechanism of estrogen but also provides crucial evidence that estrogen-induced apoptosis can be mimicked by manipulating the unfolded protein response even in breast cancer cells that are not susceptible to estrogen mediated apoptosis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-10-02

Title: Transmembrane protein 33 (TMEM33) induces apoptosis via UPR signaling and autophagy in breast cancer cells

Clarke R, Hu R, Zhang X, Hilakivi-Clarke L and Kasid U. Georgetown University, Washington, DC.

Body: Breast cancer is the most common cancer diagnosed in women. Endoplasmic reticulum stress (EnR stress) and the related unfolded protein response (UPR) are activated in breast cancer cells and can promote cell survival and endocrine resistance. TMEM33 is a novel transmembrane protein that resides in the endoplasmic reticulum (EnR) and has been shown to activate the PERK and IRE1 α branches of the UPR. However, the underlying mechanism of action of this EnR resident protein TMEM33 and the cellular functions that it regulates remain largely unknown. In this study, we show that overexpression of TMEM33 induces robust cell death in breast cancer cells. TMEM33 overexpression strongly activates UPR associated pro-death JNK-p53 signaling. We also observed a significant inhibition of the downstream survivin, which blocks cell death activation by binding to caspases and inhibiting their activation. We further show that the blockage of JNK activation with either an inhibitor or overexpression of survivin, protects cells against TMEM33 induced apoptosis. In addition, we show that TMEM33 overexpression induces autophagy in breast cancer cells. Inhibition of autophagy with using either the inhibitor chloroquine or knockdown of the Atg5 gene, further sensitizes breast cancer cells to the effects of TMEM33 overexpression. Cell death induced by TMEM33 is also decreased by overexpression of the autophagy gene Beclin 1. The findings in this study demonstrate that the novel EnR resident protein TMEM33 induces cell death by activating IRE1 α -JNK-p53-survivin signaling in breast cancer cells. Concurrently, autophagy is also activated by TMEM33, and functions as a pro-survival mechanism. Cell fate reflects the balance between the pro-death and pro-survival activities as regulated by TMEM33.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-10-03

Title: Pterostilbene enhances TRAIL-induced apoptosis in TRAIL-resistant triple negative breast cancer cells

Wu Y-C, Wang H-C, Chen C-J, Liu L-C and Way T-D. China Medical University Hospital, Taichung, Taiwan; School of Medicine, College of Medicine, China Medical University, Taichung, Taiwan and College of Biopharmaceutical and Food Sciences, China Medical University, Taichung, Taiwan.

Body: Triple-negative breast cancer (TNBC) is refractory to commonly used chemotherapeutic agents, as a result it leads to relatively poor prognosis. TNF-related apoptosis-inducing ligand (TRAIL) is currently in clinical trials as a treatment for cancer. Unfortunately, patients develop resistance to the TRAIL, therefore, agents that can sensitize cells to TRAIL are urgently needed. Pterostilbene (PTER), a natural dimethylated analog of resveratrol, is known to have diverse pharmacologic activities. In the present study, we investigated whether PTER affect TRAIL-induced apoptosis and its mechanism in TRAIL-resistant TNBC cells. First, our results indicated that PTER induced apoptosis in TNBC BT-20 cells. Next, we found that PTER enhanced TRAIL-induced apoptosis in TRAIL-resistant TNBC BT20 and MDA-MB-468 cells. We demonstrated that PTER induced both death receptor (DR)-5, DR4 and decreased decoy receptor (DcR)-2 expression. PTER also decreased the expression of anti-apoptotic proteins survivin, Bcl-xL and c-FLIP_{S/L}, but had minimal effect on the expression of Bcl-2. PTER caused the cleavage of bid protein and enhanced the expression of pro-apoptotic Bax. Moreover, we found that PTER induced DR4 and DR5 expression through the reactive oxygen species (ROS) –mediated activation of extracellular signal-regulated kinase 1/2 (ERK 1/2) and p38 mitogen activated protein kinase (p38 MAPK). Overall, our results show that PTER can potentiate TRAIL-induced apoptosis through the ROS–mediated activation of ERK 1/2 and p38 MAPK leading to DR4 and DR5 induction and down-regulation of anti-apoptotic proteins.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-11-01

Title: A genetic model of mammary gland fibrosis and cancer risk

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Body: Ductal cells of the mammary gland arise from mammary stem cells (MSC) and consist of estrogen receptor negative (ER-) luminal cells and a small ER+ luminal population. Luminal cells are maintained by the proliferation of ER- luminal progenitor (LP) cells, which are expanded in basal subtype breast cancer. Human breast LP cells exhibit telomere DNA damage, which is associated with mammographic density and increased cancer risk. Short telomeres resulting from the DNA damage response correlate with aggressive breast cancer phenotypes. Telomeric repeat factor 2 (TRF2) binds to telomeres and protects them from the DNA damage response. TRF2 expression is reduced in most human breast cancers but the phenotypic advantages of this genetic alteration are unknown. To determine the effects of telomere DNA damage on mammary gland function and tumor phenotype, we deleted TRF2 expression in mammary gland epithelium. Mammary glands lacking TRF2 expression exhibited increased telomere DNA damage response, histopathological and functional degeneration, and prominent ductal fibrosis. MSC from TRF2 deficient glands were unable to completely regenerate mammary glands following transplantation to cleared fat pads. TRF2 deficient mammary tumors also demonstrated telomere DNA damage response but exhibited rapid onset and increased proliferation. Tumor derived LP cells underwent expansion but exhibited high telomere DNA response and failed to form tumors following transplantation. In contrast, the severely depleted MSC population was highly tumorigenic and maintained telomeres via the ALT mechanism. Telomere DNA damage response in mammary tumors resulted in p53 dependent ER+ cellular differentiation and sensitivity to anti-estrogen therapy. Our results provide a new in vivo model for exploring mechanisms of mammographic density, stem cell differentiation, cancer risk, and therapeutic sensitivity.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-01

Title: Independent validation of EarlyR gene signature in BIG 1-98: A randomized, double-blind, phase III trial comparing letrozole and tamoxifen as adjuvant endocrine therapy for postmenopausal women with hormone receptor-positive, early breast cancer

Buechler S, Gray KP P, Gökmen-Polar Y, Willis S, Thürlimann B, Kammeler R, Leyland-Jones B, Badve SS S and Regan MM M. University of Notre Dame, Notre Dame, IN; IBCSG Statistical Center, Dana Farber Cancer Institute, Boston, MA; Indiana University School of Medicine, Indianapolis, IN; Avera Cancer Institute, Sioux Falls, SD; International Breast Cancer Study Group Coordinating Center and Pathology Office, Bern, Switzerland; Breast Center, Kantonsspital, St. Gallen, Switzerland and Swiss Group for Clinical Cancer Research SAKK, Berne, Switzerland.

Body: *Background:* EarlyR is a prognostic gene signature score in ER+ breast cancer (BC) computed from the expression values of *ESPL1*, *SPAG5*, *MKI67*, *PLK1* and *PGR* using a novel algorithm. EarlyR has been validated in multiple cohorts profiled on Affymetrix and Illumina microarrays. This study sought to verify prognostic features of EarlyR in a cohort of BIG 1-98.

Patients and Methods: Illumina DASL assay was used to measure gene expression in FFPE tissue of primary BC from a case-cohort sampling subset of postmenopausal women in BIG 1-98 treated with adjuvant endocrine therapy (letrozole or tamoxifen). Chemotherapy treatment was at the discretion of individual physicians and patients. Among the 1218 patients centrally reviewed with sufficient RNA material for the DASL assay, 1174 with ER+ status and assessed for EarlyR are included in the analytic cohort. EarlyR scores and pre-specified risk strata (≤ 25 =low, 26-75=intermediate, >75 =high) were computed, while blinded to clinical data. The analysis endpoints included distant recurrence free interval (DRFI) defined as time from randomization to BC recurrence at a distant site within 8 years and BC free-interval (BCFI) defined as time from randomization to first invasive BC recurrence at a local, regional or distant site or invasive contralateral BC within 8 years. Weighted proportional hazards models (univariate and multivariate, stratified by treatment assignment) were used to adjust for Kaplan-Meier, hazard ratio estimates and Wald test statistics to obtain unbiased analyses and to give consistent estimates.

Results: The distribution of the EarlyR risk groups was 67% low, 19% intermediate and 14% high risk in this ER+ cohort. EarlyR was prognostic for 8-year DRFI (P-trend=0.008). Patients with high EarlyR risk score (>75) had significantly increased risk of distant recurrence within 8 years (univariate HR=1.73, 95%CI: 1.14-2.64) compared to low EarlyR risk group (≤ 25). The estimated 8-year DRFI (95%CI) is 84%(80%-88%) for high risk vs. 91%(89%-92%) for low risk, corresponding to an absolute DRFI risk reduction of 7% (low vs high). EarlyR is also prognostic of 8-year BCFI in ER+ (P-trend=0.002) with the estimated 8-year BCFI (95%CI) 79%(75%-84%) for high risk vs. 88%(86%-89%) for low risk. Consistent results were observed in ER+, HER2- (P-trend=0.01 for DRFI, P-trend=0.004 for BCFI), in ER+, LN- (P-trend=0.05 for DRFI, P-trend=0.03 for BCFI) and ER+, LN+ (P-trend=0.08 for DRFI, P-trend=0.03 for BCFI) subsets.

Conclusions: This study confirmed the prognostic significance of EarlyR using FFPE tissue from the BIG 1-98 trial. In analyses of all ER+ patients and subsets LN-, LN+ and HER2-, EarlyR classifies 65%-70% of patients as low risk, 11-16% as high risk, and $< 20\%$ as intermediate risk. In these subsets, the size of the low risk group is larger and the size of the intermediate risk group is smaller than those reported for commercially available signatures. EarlyR identifies a set of high-risk patients with relatively poor prognosis who may be considered for additional treatment. The clinical utility of EarlyR requires further study.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-02

Title: Serum-triglycerides among triple negative breast cancer patients as a biomarker of poor outcome

Lofterød T, Mortensen ES S, Nalwoga H, Wilsgaard T, Frydenberg H, Risberg T, Eggen AE, McTiernan A, Aziz S, Wist EA A, Reitan JB B, Akslen LA A and Thune I. Oslo University Hospital, Oslo, Norway; University Hospital of North Norway, Tromsø, Norway; University of Bergen, Bergen, Norway; UIT The Arctic University of Norway, Tromsø, Norway; University Hospital of North Norway, Tromsø, Norway; Fred Hutchinson Cancer Research Center, Seattle; Haukeland University Hospital, Bergen, Norway and UIT The Arctic University of Norway, Tromsø, Norway.

Body: Background: Obesity and related metabolic imbalances, including increased activity of free fatty acids, may promote tumor growth and metastasis. Fatty acids are mainly stored as triacylglycerols. Yet, the role of serum-triglycerides on breast cancer prognosis is still undefined.

Methods: A population based survival study among 575 breast cancer patients identified within the Tromsø study during 1979-2008, was conducted. Pre-diagnostic serum triglycerides, high density lipoprotein-cholesterol, total cholesterol, height and weight were measured. Histopathological and clinical data were obtained from medical records, and hormone receptor, HER2 status, and Ki-67 were re-analyzed on tissue microarray blocks. Multivariate Cox proportional Hazard regression models were used to study the associations between patient characteristics including s-triglycerides, and breast cancer survival.

Results: Among 575 women with invasive breast cancer (stage 1-3), a total of 87 women were diagnosed with triple negative breast cancer (TNBC). Patients diagnosed with TNBC, compared to non-TNBC, were likely to be younger at diagnosis (55.3 vs 57.9 years, $p=0.061$), they had larger tumors (29.7 mm vs 22.5 mm, $p=0.001$), and higher Ki-67 (31.1% vs 15.9%, $p<0.001$). After a mean follow-up of 8.4 years, TNBC patients with above median levels of s-triglycerides ($> 0.98\text{mmol/L}$) compared to TNBC patients with below median levels of s-triglycerides ($\leq 0.98\text{mmol/L}$) had 3.0 times higher risk for breast cancer recurrence or breast cancer specific death (HR 3.02, 95% CI 1.21-7.55), and 3.4 times higher overall mortality risk (HR 3.41, 95% CI 1.38-8.45). Among the TNBC patients, women with above median s-triglycerides had 15% lower 5-year disease-free survival (76% vs 91%) and 18% lower 5-year overall survival (74% vs 92%) compared to women with below median s-triglycerides.

Conclusions: Our results strongly support s-triglycerides as an important biomarker for breast cancer outcomes among triple negative breast cancer patients.

Table 1: Multivariable adjusted Hazard Ratios (HRs) for incidence breast cancer recurrence or breast cancer specific death, and incidence overall mortality by pre-diagnostic serum-triglycerides among non-triple negative breast cancer (TNBC) and TNBC patients

	Non-TNBC, n=488			TNBC, n=87		
		Recurrence or breast cancer specific death (n=90)	Overall mortality (n=104)		Recurrence or breast cancer specific death (n=24)	Overall mortality (n=33)
	n	HR (95% CI)	HR (95% CI)	n	HR (95% CI)	HR (95% CI)
s-Triglycerides						
Median						
$\leq 0.98 \text{ mmol/l}$	257	1.00	1.00	43	1.00	1.00
$> 0.98 \text{ mmol/l}$	231	0.87 (0.56-1.35)	1.06 (0.70-1.62)	44	3.02 (1.21-7.55)	3.41 (1.38-8.45)
Tertiles						
$\leq 0.82 \text{ mmol/l}$	173	1.00	1.00	26	1.00	1.00
0.83 – 1.22 mmol/l	166	0.74 (0.44-1.23)	0.81 (0.49-1.36)	31	1.37 (0.38-4.98)	0.98 (0.31-3.08)
$\geq 1.23 \text{ mmol/l}$	149	0.88 (0.50-1.54)	1.17 (0.66-1.96)	30	6.63 (1.64-19.3)	3.87 (1.52-12.0)

p-trend		0.495	0.357	0.005	0.007

Multivariate Cox proportional Hazard regression model. Adjusted for BMI and age at attendance (continuous), age at diagnosis (continuous), breast cancer stage at diagnosis (categorical), and current smoking (categorical). Abbreviation: CI, confidence interval; n, number of cases; TNBC, triple negative breast cancer

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-03

Title: Estrogen receptor-positive breast cancer in *BRCA1* mutation carriers shows a BRCAness profile, suggesting sensitivity to drugs targeting homologous recombination deficiency

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Body: *Background*

As estrogen receptor (ER)-positive breast cancer in *BRCA1* mutation carriers arises at an older age with less aggressive tumor characteristics than ER negative *BRCA1* mutated breast cancer, it has been suggested that these tumors are 'sporadic' and not *BRCA1*-driven. With the introduction of targeted treatments specific for tumors with a non-functioning *BRCA1* or *BRCA2* gene, the question whether the BRCA genes are impaired in the tumor, is highly relevant. Therefore, we performed genomic profiling of *BRCA1*-mutated ER+ tumors.

Methods

Genomic profiling, BRCA1 promoter methylation assessment, and loss of heterozygosity (LOH) analysis were done on 16 *BRCA1*-mutated ER+ tumors. Results were compared with 57 *BRCA1*-mutated ER- tumors, 36 *BRCA2*-mutated ER+ associated tumors, and 182 sporadic ER+ tumors.

Results

The genomic profile of *BRCA1*-mutated ER+ tumors was different from *BRCA1*-mutated ER- breast tumors, but highly similar to *BRCA2*-mutated ER+ tumors. In 83% of the *BRCA1*-mutated ER+ tumors, loss of the wildtype *BRCA1* allele was observed. Clinico-pathological variables in *BRCA1*-mutated ER+ cancer were more similar to *BRCA2*-mutated ER+ and sporadic ER+ breast cancer than to *BRCA1* mutated ER- cancers.

Conclusions

As *BRCA1*-mutated ER+ tumors showed a BRCAness copy number profile and LOH, it is likely that the loss of a functional *BRCA1* protein plays a role in tumorigenesis in *BRCA1*-mutated ER+ tumors. Therefore, we hypothesize that these tumors are sensitive to drugs targeting the *BRCA1* gene defect.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-04

Title: Breast cancer subtype, age and lymph node status as predictors of local recurrence following breast-conserving therapy

Braunstein LZ Z, Taghian AG G, Niemierko A, Salama L, Capuco A, Wong JS S, Punglia RS S, Bellon JR R, MacDonald SM M and Harris JR R. Harvard Radiation Oncology Program; Dana Farber Cancer Institute and Massachusetts General Hospital.

Body: Purpose/Objectives: Advances in breast-conserving therapy (BCT) have yielded local control rates comparable or superior to those of mastectomy. Here, we sought to identify risk factors associated with isolated local recurrence (LR) following BCT.

Materials/Methods: This study included a multi-institutional cohort of 2,233 consecutive breast cancer patients who underwent BCT between 1998 and 2007. Patient characteristics and disease parameters were stratified by age, subtype and nodal status. Biologic subtype was approximated by receptor status and tumor grade. No patients received HER2/neu-directed therapy. The association of clinicopathologic features with LR was evaluated using Cox proportional hazards regression models.

Results: At a median follow-up of 106 months, 69 LR events (3.1%) were observed. Among the overall cohort, 10-year freedom from LR was 95.9%. On univariate Cox regression analysis, risk factors associated with LR included subtype other than luminal A (hazard ratio [HR] for luminal B = 3.01, HER2 = 6.29, triple negative [TNBC] = 4.72; $p < 0.001$ for each), younger age (HR of oldest versus youngest quartile = 0.43; $p = 0.005$), regional lymph node involvement (HR for 4-9 involved nodes = 3.04; > 9 nodes = 5.82; $p < 0.01$ for each), positive resection margins (HR = 2.43; $p = 0.005$), and high-grade disease (HR = 5.37; $p < 0.001$). Presence of LVI (HR = 1.56; $p = 0.06$) or 1-3 involved nodes (HR = 1.55; $p = 0.07$) approached significance. Multivariate Cox regression demonstrated an association with LR among those with non-luminal A subtypes (HR for luminal B = 2.64, HER2 = 5.42, TNBC = 4.32; $p < 0.001$ for each), younger age (HR for age > 50 = 0.56; $p = 0.01$), and any nodal disease (HR = 1.06 per involved node; $p < 0.004$).

Conclusions: BCT yields favorable outcomes for the large majority of patients, although increased LR was observed among those with non-luminal A subtypes, younger age, and increasing lymph node involvement. Risk factors for LR following BCT appear to be converging with those following mastectomy in the current era.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-05

Title: Extracellular vesicles from young women's and postpartum breast cancer display unique proteomic content, alter breast cancer aggressive behavior, and influence immune cell function

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Body: Background: Young women's breast cancer [YWBC] affects 27,000 US women age ≤ 45 annually. Half of these cancers occur within 5-10 years of a prior childbirth, a postpartum breast cancer [PPBC], incurring a 3 fold increased risk for metastasis and death. Recently, extracellular vesicles [EV] have been identified in human circulation, released from cancer cells, that have paracrine and autocrine effects, alter the tumor microenvironment and establish metastatic niches. EVs isolated from breast cancer lines increase proliferation and invasion of other breast cancer cells *in vitro*. However, the impact of EVs isolated from primary breast cancer patients on tumor invasion, metastasis and their role in tumor immune suppression is largely unknown. We hypothesized that EVs from YWBC/PPBC patients may contain unique pro-metastatic cargo, influence aggressive breast cancer cell behavior and may demonstrate the ability to alter immune cell function.

Method: We isolated EVs using size-exclusion chromatography [SEC] from the plasma of 10 unaffected young women and 20 YWBC patients balanced for parity, age, subtype and stage. We compared the breast cancer-specific EV proteins within various clinical groups of YWBC and PPBC to identify significant proteomic differences by parity, sub-type, stage, and disease recurrence. We determined the functional impact of these EVs on tumor cell motility and proliferation, and analyzed the effect of breast cancer derived EVs on immune cell phenotype, function, and T cell proliferation assays.

Results: Of the 582 proteins, 22 proteins are significantly increased in the EVs of YWBC compared to unaffected donors. The protein set includes breast cancer antigens [MUC 1, 2, 5b], transcriptional regulators [Myc target protein], enzymes [catalase, MMP inhibitor 1], and signaling molecules [Annexin 1, latent TGF β binding protein 1], among others. Several identified proteins specifically track with those YWBC cases with subsequent metastases. Furthermore, 8 unique proteins track with PPBC, including cartilage oligomeric matrix protein, a novel breast cancer biomarker that correlates with increased invasiveness, and decreased recurrence-free survival. EVs isolated from the plasma of newly diagnosed YWBC increase breast cancer invasion and EVs derived from breast cancer are engulfed by the majority of monocytic immune cells, including dendritic cells, classical and activated monocytes, but not by lymphocytes. Specifically, CD14 $^{+}$ monocytic myeloid derived suppressor cells engulfed the EVs while the CD15 $^{+}$ granulocytic subset did not. Once engulfed, phenotypic changes occur in the EV containing monocytes and a significant reduction in T cell stimulation in standard mixed-lymphocyte reactions is observed.

Conclusion: EVs isolated from YWBC & PPBC cases have unique protein content and increase breast cancer invasiveness, which suggests potential mechanistic roles for EVs as increasing metastatic risk and provides novel candidate biomarkers. We identified an immunomodulatory effect of breast cancer EVs on human monocytes that may contribute to immunosuppression in breast cancer and a role for EVs as directly modulating the host and tumor microenvironment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-06

Title: Quantification of HER2-driven signaling (HER2_S) inhibition of four different anti-HER2 drugs tested *ex vivo* in live primary HER2-negative breast cancer cell samples with abnormal HER2 signaling activity

Laing L, Burns D, Huang Y, MacNeil I, Rich B, Myhre S, Soltani S and Sullivan B. Celcuity, Minneapolis, MN.

Body: Background: A new functional cellular analysis platform, the CELx HER2 Signaling Profile (CELx HSP) Test, uses a label-free impedance biosensor to measure HER2 signaling activity in live tumor cells. A recently completed study quantified HER2-driven signaling activity in epithelial cell samples extracted and cultured from fresh breast tissue specimens obtained from 34 patients with HER2-negative breast cancer (DAKO 0 or 1+). Of the cell samples tested, 7 of 34 HER2-negative breast tumor patients (20.5%; 95% CI=10%-37%) were found to have abnormal HER2 signaling activity (HER2_S+). The current study set out to: 1) evaluate the primary cells with abnormal HER2-driven signaling with four HER2 signal inhibitors - pertuzumab, lapatinib, neratinib, afatinib; and 2) evaluate the same four HER2 signal inhibitors with 9 HER2-positive cell lines. The objective was to quantify the percentage of HER2-driven signaling activity each drug could inhibit *ex vivo* in the primary cell samples and cell lines. Comparing the results between the HER2-negative primary cells and the HER2-positive cell lines was also of interest. The anti-HER2 drug, trastuzumab, was not studied because its primary mechanism of action does not appear to be direct mediation of HER2-driven signaling.

Methods: Epithelial cells from the 7 HER2-negative tumor specimens with abnormal HER2-driven signaling (HER2_S+) and the 9 HER2-positive cell lines were obtained. Real time live cell response to NRG1, a specific HER2/HER3 agonist, with or without a HER2 targeted drug (pertuzumab, lapatinib, neratinib, afatinib) was measured and quantified using an xCELLigence RTCA impedance biosensor (ACEA Biosciences, San Diego, CA). Clinically relevant concentrations of the HER2 drugs were used. From these responses, the percentage inhibition of the HER2-driven signaling initiated by NRG1 by the HER2 drugs was determined.

Results: Each of the HER2 drugs inhibited an average of at least 69% of the HER2-driven signaling activated by NRG1 stimulation in the HER2-negative primary cell samples; the highest level of inhibition was found with the two irreversible covalent dual RTKi's, afatinib and neratinib. All of the HER2 drugs inhibited a greater percentage of HER2-driven signaling in the HER2-negative primary tumor cells than in the HER2-positive cell lines.

table 1

HER2 Drugs	Mechanism of Action	Avg. % NRG1 Inhibition	
		Cell Lines (HER2+)	Primaries (HER2-)
Pertuzumab	HER2 dimerization inhibitor	46%	78%
Lapatinib	Reversible Dual RTKi (HER2, EGF)	15%	69%
Afatinib	Irreversible Covalent Dual RTKi (HER2, EGF)	47%	93%
Neratinib	Irreversible Covalent Dual RTKi (HER2, EGF)	95%	100%

Conclusions: These findings provide strong evidence that HER2 signal inhibitors are effective in blocking abnormal levels of HER2-driven signaling (HER2_S+) *ex vivo* in live primary cells from breast cancer patients with normal expression levels of HER2. These results suggest a new group of breast cancer patients, HER2-negative with abnormal HER2 signaling (HER2-/HER2_S+), may benefit from the addition of HER2 signal inhibitors to current combination therapeutic regimens. Additional studies to confirm these findings are underway.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-07

Title: *CCND1* amplification in early breast cancer patients: Correlation with subtypes and prognosis

Hanf DC C, Fasching PA A, Villalobos IE E, Gasparyan A, Wachter D, Santiago A, Guzman R, Weihbrecht S, Hanf V, Hartmann A, Beckmann MW W and Press MF F. University Hospital Erlangen, Comprehensive Cancer Center Erlangen-EMN, Erlangen, Germany; Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA; Breast Center Klinikum Fuerth, Fuerth, Germany and Institute of Pathology, University Hospital Erlangen, Erlangen, Germany.

Body: Background: Mechanisms of progress and recurrence of early breast cancer (BC) have gained importance since several targeted therapeutic options in metastatic breast cancer have been introduced over the last years, especially in hormone receptor positive BC. This study investigates the amplification of cell cycle regulator cyclin D1 gene (*CCND1*) amplification as one possible progression mechanism.

Patients & Methods: Patients from an unselected cohort of early BC patients were included into this study. A tissue microarray (TMA) was available for n=832 patients with early breast cancer. *CCND1* amplification was assessed after FISH (Abbott Vysis LSI *CCND1* SpectrumOrange/CEP11 SpectrumGreen Probes Kit). A *CCND1*/CEP11-ratio ≥ 2.0 was considered as amplification. Staining was successful in 545 tumor cores. Amplification results were correlated with clinical patient and tumor characteristics and survival analyses were performed with regard to distant disease free survival and overall survival.

Results: *CCND1* amplification was found in 13.6% of patients. Triple negative, luminal A like, luminal B like and HER2 positive tumors were amplified in 7.5%, 7.8%, 18.6% and 15.7% respectively ($p = 0.010$). *CCND1* amplification was significantly associated with a higher grading and a higher body mass index. Furthermore an amplification was seen more frequently in lobular BC and ductal BC than other histological subtypes.

Survival analysis showed a reduced DDFS for patients with *CCND1*-amplification. 5 year DDFS rates were 90.6% for non-amplified tumors and 86.0% for amplified tumors ($p, \log\text{-rank} = 0.066$). 5 year OS rates were 93.0% for non-amplified tumors and 90.1% for amplified tumors ($p, \log\text{-rank} = 0.119$).

Adjusted for age, tumor size, nodal status and molecular subtype, cox regression showed HR of 1.3 (95% CI: 0.76-2.5, $p=0.46$) for DDFS and a HR of 1.33 (95% CI: 0.7-2.53, $p=0.38$) for OS.

Conclusion: With a 13.6% prevalence in all breast cancer patients, mainly present in luminal B like cancers, *CCND1*-amplification is a genetic aberration associated with an unfavorable prognosis. Within hormone receptor positive women *CCND1* amplification might play a role in treatment resistance mechanisms in early breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-08

Title: Use of an aptamer library based next generation omics platform for the development of a novel trastuzumab predictive assay

Spetzler D, Domenyuk V, Santhanam R, Wei X, Stark A, Wang J, Gatalica Z, Miglarese M, Vidal G and Schwartzberg LS S. Caris Life Sciences, Phoenix, AZ and The University of Tennessee Health Science Center, Memphis, TN.

Body: Introduction: Previous attempts to use individual aptamers as diagnostic reagents have failed to consistently achieve performance comparable to antibodies. Here we report a novel systems biology approach using poly-ligand aptamer libraries to identify responders and non-responders to trastuzumab-based regimens in metastatic breast cancer.

Methods: To overcome the fundamental limitation of the individual aptamer binding affinities, large libraries (10^6 species) were created so that potentially thousands of aptamers could bind to each of a multitude of targets related to the whole cellular changes in response to trastuzumab therapy. A set of breast cancer patients, which received trastuzumab mono- or combined therapy for at least 7 months were classified as "Responders" (R); cases with particular regimen discontinued in the period not exceeding 5 months were classified as "Non-Responders"(NR). A library of 2×10^{12} unique 90-mer ssDNA oligodeoxynucleotides (ssODN) was trained on FFPE tissue of both R and NR patients. Partitioning of aptamer libraries was done by microdissection of the tumor tissue, after incubation of aptamer library with the entire tissue section, to drive selection pressure toward cancer cells. A total of 10 cases of R and NR, 6 Her2+ cases each, were used to train separate aptamer libraries, with 1 positive and 2 counter selection cases per enrichment. Enriched libraries were screened on 20 R and 20 NR cases (11 Her2+ cases each) by adopting modified immunohistochemistry protocol. Each library was used as an independent reagent (similar to an antibody in IHC) across all 40 cases to evaluate the efficacy of the aptamer library to distinguish differences between the R and NR groups. Staining (DAB chromogen) profiles were scored from 0 to 3+ (nuclear and cytoplasmic staining) by a pathologist without any knowledge of the clinical outcomes. Initial validation was done by t-test using raw histological scores. Four libraries showed significant p-values between groups of responders and non-responders, a classification algorithm was constructed and evaluated using area under the receiver-operator characteristic curve (AUC). The datasets of two best-performing libraries were combined into one model using logistic regression to further improved the classifier performance.

Results: Of seventeen trained libraries, eight were evaluated and four showed significant correlation to clinical benefit with a minimum accuracy of 75% for each library when evaluated independently. Furthermore, two libraries showed exceptional performance (ROC curve AUC of 0.86 and 0.77). Combination of the profiling data from these two libraries using logistic regression resulted in an AUC of 0.985. A prospective validation of aptamer histochemical theranostic testing has been initiated.

Summary: Enriched aptamer libraries appear to distinguish trastuzumab responsiveness in metastatic breast cancer. This technology could be used as an additional technique beyond FISH testing to determine sensitivity to anti-HER2 agents. The demonstrated platform is applicable to virtually any disease where the safe and effective use of corresponding drug is yet to be improved.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-09

Title: The immune response in triple negative breast cancer

Gillgrass AE E, Pond GR R, Levine MN N, Whelan TJ J, Hassell JA A and Bane AL L. McMaster University, Hamilton, ON, Canada.

Body: Background: Triple Negative Breast Cancer (TNBC) is often associated with a poor prognosis. However TNBC is a heterogeneous group of tumors and while some patients have a poor prognosis others appear to do well long-term. There are currently no clinical or pathologic tumor features that distinguish poor from good outcome. Some TNBCs have infiltration with immune cells and the degree of this infiltration correlates with prognosis.

Objectives: 1) To comprehensively examine immune factors associated with outcome in a cohort of TNBC patients, and 2) to develop an immune gene signature that can stratify patients into low and high risk groups.

Methods: We profiled RNA from 22 TNBCs (10 who had experienced a recurrence) from our institutional cohort using the PanCancer Immune Profiling Panel from NanoString. This panel consists of an extensive list of 770 genes designed to evaluate the immune microenvironment of tumors. The genes fall into a number of functional categories including; 1. Genes that identify specific immune cells, 2. Cytokines that promote an effective immune response and others that are associated with immunosuppression, 3. Chemokines, which attract immune cells into the tumor, 4. Genes that assess both the activation and inhibition of immune cell function, 5. Genes that identify tumor specific antigens. Analysis was performed in the nSolver Advanced Analysis Program.

Results: Using unsupervised hierarchical clustering of genes that were highly differentially expressed, the tumors were classified into 3 immune groups with distinct clinical outcomes. Group 1 (*'Immune Excluded'*), consisted of tumors with the lowest levels of expression of the immune markers assessed, suggesting that these tumors have a low or absent immune infiltrate; 7 of 7 patients in this group recurred. Group 2 (*'Immune Activated'*) contains tumors that had the highest levels of anti-tumoral immune cell genes and their activation markers. This we interpret to represent a tumor group with a robust anti-tumor immune response; 0 of the 6 patients in this group recurred. In comparison Group 3 (*'Immune Low'*) had moderate to low levels of expression of the majority of immune genes assessed. This we interpret to represent a group of tumors with limited immune cells present; 3 of 9 patients in this group recurred. Lastly, when comparing scores for immune cells, patients that recurred had lower scores for cytotoxic cells, CD8 T cells, Th1 cells and B cells.

Conclusion: In this pilot study high expression of anti-tumoral immune genes correlated with good outcome, whereas lower/absent expression of these genes correlated with poor outcome. We are currently extending these findings to our entire cohort of 180 TNBC patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-10

Title: Prognostic relevance of caspase 8 polymorphisms for breast cancer

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Body: Background: The minor allele of two caspase 8 polymorphisms, namely *CASP8* -652 6N InsDel (rs3834129) and *CASP8* Asp302His (rs1045485), were repeatedly associated with reduced breast cancer susceptibility. Contrarily, the presence of the -652 6N Del or the *CASP8* 302His variant was reported to be an unfavorable prognostic factor in colorectal cancer or neuroblastoma. However, prognostic relevance of these genetic variants for breast cancer is completely unknown and is therefore addressed by the current study.

Methods: Genotyping was performed by pyrosequencing. Caspase 8 mRNA expression was quantified by comparative RT-qPCR.

Results: We observed an allele-dose dependent association between *CASP8* -652 6N InsDel and caspase 8 mRNA expression in breast cancer tissue, with homozygous deletion carriers showing lowest relative caspase 8 expression ($p=0.0131$). Intriguingly, the presence of the -652 6N Del or the 302His variant was shown to be a negative prognostic factor for breast cancer in terms of an allele-dose dependent influence on overall survival (OS, $p=0.0018$, $p=0.0150$, respectively). Moreover, both polymorphisms were independent predictors of OS after adjusting for co-variates ($p=0.007$, $p=0.037$, respectively). Prognostic relevance of both polymorphisms was independent from ER or Her2/Neu receptor status and a combined analysis of diplotypes revealed an additive influence on OS ($p=0.0002$).

Conclusion: This is the first report, showing negative and independent prognostic impact of the *CASP8* -652 6N Del and the 302His variant for breast cancer. Our data provide rationale to further validate clinical utility of these polymorphisms for breast cancer and to extend this investigation to a broad scope of other malignancies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-11

Title: Up-regulation of SPARC is associated with breast tumor progression and epithelial SPARC expression is correlated with poor survival and MMP-2 expression in patients with breast carcinoma

Lee JS, Kim G-E, Park MH and Yoon JH. Chonnam National University Medical School, Gwangju, Republic of Korea and Chonnam National University Medical School, Gwangju, Republic of Korea.

Body: Background: Secreted protein acidic and rich in cysteine (SPARC) plays a crucial role in the process of tumor invasion and metastasis in many cancers. Matrix metalloproteinases (MMPs) degrade the extracellular matrix and participate in several key processes of invasion and metastasis. The aims of this study were to evaluate the potential involvement of SPARC in the progression of breast tumor and to determine its association with outcome variables and MMPs expression in patients with breast carcinoma (BC).

Materials and Methods: SPARC expression was examined in 8 pairs of BC tissues and surrounding normal tissues at mRNA and protein levels by quantitative real-time PCR (qRT-PCR), RNAscope in situ hybridization (ISH), Western blotting, and immunohistochemistry techniques. Immunohistochemical staining of SPARC on tissue microarray was done in 26 normal breasts, 76 ductal carcinoma in situ (DCIS), and 198 BC samples. In addition, we performed immunohistochemical staining for MMP-2 and MMP-9 in BC.

Results: SPARC expression at mRNA and protein levels by qRT-PCR and Western blotting was significantly increased in BC tissues compared to the surrounding normal tissues ($p < 0.05$ and $p < 0.01$, respectively). RNAscope ISH and immunohistochemistry of SPARC confirmed that SPARC expression was increased in BC tissues compared with their normal tissues and its expression was more pronounced in the stromal compartment than in epithelial compartment. SPARC expression was different among the normal, DCIS and BC groups and epithelial SPARC expression increased progressively from normal breast through DCIS to BC ($p < 0.001$). In patients with BC, high epithelial SPARC expression was associated with worse disease-free and overall survival ($p = 0.002$ and $p = 0.048$, respectively) and independently predicted worse disease-free survival ($p = 0.002$). Epithelial SPARC expression was significantly correlated with MMP-2 expression ($p < 0.05$).

Conclusion: Our results suggest that up-regulation of SPARC contributes to breast tumor progression. SPARC expression may be a useful biomarker for the prognostic prediction in patients with BC. SPARC can control extracellular matrix degradation through up-regulation of MMP-2.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-12

Title: Quantitative *in vivo* imaging of intratumoral heterogeneity to assess tumor response to trastuzumab treatment in a preclinical model of HER2+ breast cancer

Syed A, Quarles CC, McIntyre JO, Barnes SL L, Yankeelov TE E and Sorace AG G. University of Texas, Austin, TX and Vanderbilt University, Nashville, TN.

Body: Introduction:

Intratumoral heterogeneity is associated with variable treatment response and poorer patient prognosis. Initial changes in intratumoral cellular and vascular heterogeneity after treatment could serve as an early metric for treatment response and be used to guide and optimize therapy for improved patient outcome. Quantitative, noninvasive imaging can characterize, in 3D, tumor response to treatment by evaluating longitudinal alterations in heterogeneity. Our goal is to quantitatively image the changes in intratumoral cellular and vascular heterogeneity in response to trastuzumab in a murine model of HER2+ breast cancer.

Experimental Design:

Data from two independent cohorts of mice were used in this study. Mice were implanted subcutaneously with BT474 breast cancer cells (1×10^7) and randomly assigned to trastuzumab treated (10 mg/kg) or saline control groups. After tumors reached $\sim 225 \text{ mm}^3$, intraperitoneal injections of trastuzumab or saline were given on days 0 and 3. In the first cohort of mice ($n=14$), quantitative dynamic contrast enhancement magnetic resonance imaging (DCE-MRI) assessed tumor response on days 0 (prior to treatment), 1 and 4. In this study, we focused on the calculated extravascular extracellular space, measured using DCE-MRI parameter, v_e . In the second cohort of mice ($n=10$), ^{18}F -flouromisonidazole positron emission tomography (FMISO-PET) imaging was utilized to assess tumor hypoxia on days 0 (prior to treatment), 1, 3, 4, and 7 through changes in standardized uptake value (SUV). Longitudinal changes in heterogeneity were assessed using voxel based histogram analysis of v_e or SUV. Statistical analysis (R Studio) compared histogram analyses (full-width half-maximum, range, kurtosis, and standard deviation) of control and treated groups. Tumor size measurements obtained at the conclusion of the experiment were used to validate imaging findings of tumor response to treatment.

Results:

DCE-MRI revealed increased heterogeneity in extravascular extracellular space after trastuzumab treatment, as quantified by a significant increase in the FWHM of v_e voxel distributions at day 4 post-treatment ($p=0.03$). Additionally, the FMISO-PET data showed a significant decrease in hypoxic regional heterogeneity in treated tumors, as quantified by percent change (normalized to baseline) in standard deviation and range of SUV tumor voxel distributions on days 3 ($p=0.02$), 4 ($p=0.01$), and 7 ($p<0.001$) compared to saline controls.

Conclusion:

Trastuzumab improved oxygen delivery throughout the tumor as quantified by decreased hypoxia heterogeneity (narrowing of distributions) of the FMISO-PET SUV map. Likewise, the increased heterogeneity (widening of distributions) of the DCE-MRI v_e parametric map, reflects a more heterogeneous cellularity interpreted to arise from enhanced cell death following trastuzumab treatment. Ongoing analysis of heterogeneity of corresponding histology slides will be used to validate the imaging measures of heterogeneity. DCE-MRI and FMISO-PET reveal quantitative longitudinal changes in tumor cellular and vascular heterogeneity, and may be able to predict response and guide therapy for patients with HER2+ breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-13

Title: High intratumoral and stromal S100A8 expression is prognostic of poor outcome in breast cancer

Miller P, Kidwell K, Thomas D, Sabel M, Rae J, Hayes DF F, Lippman ME E and El-Ashry D. University of Miami Sylvester Comprehensive Cancer Center, Miami, FL and University of Michigan Comprehensive Cancer Center, Ann Arbor, MI.

Body: Background: S100A8 and S100A9 are members of a family of calcium binding proteins that regulate inflammatory response, and are biomarkers of inflammatory diseases, S100A8/A9 preferentially form heterodimers that interact with their receptor, RAGE, to activate signaling pathways (ERK1/2 MAPK, JNK, and NF- κ B) and stimulate tumor cells. Elevated expression of S100A8/A9 has been observed in cancers of the bladder, esophagus, colon, ovary, and breast. S100A8/A9 are expressed intratumorally by cancer cells and in the stroma by infiltrating immune and myeloid cells as well. We investigated the associations of elevated expression of intratumoral and stromal S100A8 with survival outcomes in breast cancer.

Methods: Tissue microarrays (TMA) were constructed from breast cancer specimens from patients with stage I-III breast cancer treated at the University of Michigan Comprehensive Cancer Center between 2004-2006, ensuring a minimum of 10-year follow-up. Each patient was represented on the TMA by representative regions of non-necrotic tumor and distant normal tissue. Automated Quantitative Immunofluorescence (AQUA) was performed for S100A8 protein, and samples were scored for intratumoral and stromal S100A8 expression. S100A8 staining was assessed as a continuous value and by exploratory dichotomous cutoffs. Associations with disease-free survival (DFS) or overall survival (OS) and S100A8 expression, either as continuous value or based on the exploratory cutoffs, were determined using the Kaplan-Meier method and Cox proportional hazards models.

Results: In the entire patient cohort, high intratumoral S100A8 expression, as a continuous measure, was a significant prognostic factor for OS (univariable hazard ratio [HR] 1.26, 95% confidence interval [CI] 1.02-1.56, $p=0.036$), and for DFS (multivariable HR [95%CI] = 1.24 [1.01-1.53], $p = 0.043$). Exploratory analyses demonstrated optimal cutoffs of intratumoral and intrastromal staining that greatly separated survival curves. We evaluated whether the prognostic significance of S100A8 expression is different in breast cancer patients based on hormone receptor status and determined that neither intratumoral nor stromal S100A8 expression were significantly associated with outcomes.

Conclusions: Elevated intratumoral and stromal expression of S100A8 are significant indicators of poor outcome in breast cancer patients. These data further support a biological role for S100A8 signaling in mammary carcinogenesis and aggressive tumor behavior. Evaluation of S100A8 protein expression might provide additional prognostic information beyond traditional breast cancer prognostic biomarkers. Further validation is necessary to investigate these findings.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-14

Title: Folate receptor alpha (FOLR1) expression in triple-negative breast cancer (TNBC)

Cui X, McIntire PJ J, Ginter PS S, Irshaid L, Chen Z and Shin SJ J. Weill Cornell Medicine, New York, NY.

Body: Background Folate receptor alpha (FOLR1) has been identified as a potential prognostic and therapeutic target in breast cancer. The limited studies evaluating the role of FOLR1 in breast cancer have shown that FOLR1 protein expression is enriched in triple-negative breast cancer (TNBC). Newly developed anti-FOLR1 therapy could potentially be used for patients with TNBC for whom few therapeutic options exist. We sought to evaluate FOLR1 protein expression in a cohort of patients with TNBC to determine its prevalence and prognostic value. **Design** Immunohistochemistry was performed for FOLR1 (26B3.F2, Biocare, RTU) on tissue microarray (TMA) slides consisting of 62 primary TNBC. Membranous staining in $\geq 5\%$ of cells was deemed positive in a given case. Statistical analyses correlating FOLR1 protein expression with clinicopathologic parameters and clinical outcome [disease-free survival (DSF) and overall survival (OS) (range: 16 to 196 months, mean: 111 months)] were performed.

Results 62 cases of primary TNBC from 61 patients were studied. All patients were female and the mean age was 55 years (range 30 to 91 years). Histologically, almost all tumors were invasive ductal carcinoma (94%; 58/62) and grade 3 (89%; 55/62). Most were pT1 tumors (71%; 44/62) or pT2 (27%; 17/62). For the majority of patients, the nodal status was N0 (61%; 38/62) or N1 (24%; 15/62) and the stage was 1 (53%; 33/62) or 2 (32%; 20/62). Most cases were negative for FOLR1 (84%; 52/62). FOLR1 expression did not correlate with any clinicopathologic parameters, DFS or OS ($P > 0.05$). **Conclusion** Similar to other studies, we found no correlation with FOLR1 expression and common clinicopathologic parameters and clinical outcome in TNBC. While FOLR1 expression has been previously reported to confer poor prognosis in breast cancer (all types), our findings suggest that in TNBC specifically, FOLR1 expression is not prognostically significant.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-12-15

Title: Triple-negative breast cancers (TNBCs) rich in infiltrating stromal plasma cells have improved disease-free survival and overall survival

McIntire PJ J, Cui X, Ginter PS S, Irshaid L, Chen Z and Shin SJ J. Weill Cornell Medicine, New York, NY.

Body: Background Tumor-infiltrating lymphocytes (TILs) have emerged as a prognostic indicator in patients with TNBC. Furthermore, lymphocyte-predominant breast cancer (LPBC), defined as those containing $\geq 50\%$ stromal TILs has been shown to be associated with better prognosis. While cell-mediated immunity has received much of the credit for anti-tumor responses, the International TILs Working Group recommends that the assessment of stromal density of TILs should incorporate all mononuclear cells including plasma cells. Very little is known about the role of humoral immunity, particularly the role of plasma cells in the anti-tumoral response in breast cancer. We sought to evaluate the prognostic value of plasma cells in the setting of stromal TIL assessment of TNBC. **Design** Morphologic assessment of TILs was performed on a representative H&E slide of 76 cases of primary TNBC. For each case, the stromal density of TILs was estimated and then classified as LPBC or non-LPBC using a cutoff of $\geq 50\%$ for the former. Also, the plasma cell intensity defined as the mean percentage of stromal plasma cells of five 40x high power microscopic fields of TILs was recorded at 5% increments. Statistical analyses were performed using disease-free survival (DFS) and overall survival (OS) (range: 16 to 196 months, mean: 110 months) as primary endpoints. **Results** Plasma cell intensity was dichotomized at 12.5% and predictive of OS [.21, 95% confidence interval (CI) .05-.93, $P = 0.0401$]. A positive dose related response trended toward significance for each 5% increase in plasma cell intensity (.97, 95% CI .93-1.01, $P = 0.1168$). Disease-free survival also trended positively towards significance (.39, 95% CI .13-1.20, $P = 0.0991$). When comparing LPBC, tumors rich in plasma cells ($>12.5\%$ of TILs) trended towards longer DFS (.17, 95% CI .02-1.62, $P = 0.1226$) when compared to plasma cell poor LPBC. **Conclusion** In addition to the cell-mediated immune response in TNBC, our findings suggest that humoral immunity may also play a role in predicting recurrence and survival in these patients. Our findings suggest that inclusion of an additional parameter of plasma cell intensity may be prognostically valuable in TIL assessment of TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-13-01

Title: Prospective association between breast cancer risk and an individual dietary index based on the British Food Standards Agency nutrient profiling system

Deschasaux M, Julia C, Zelek L, Kesse-Guyot E, Gourlet V, Lécuyer L, Méjean C, Ducrot P, Peneau S, Latino-Martel P, Fézeu L, Fassier P, Hercberg S and Touvier M. Sorbonne Paris Cité Epidemiology and Statistics Research Center (CRESS), Inserm U1153, Inra U1125, Cnam, Paris 13 University, Nutritional Epidemiology Research Team (EREN), Bobigny, France; Avicenne Hospital, APHP, Bobigny, France and Avicenne Hospital, APHP, Bobigny, France.

Body: Background: The Food Standards Agency Nutrient Profiling System (FSA-NPS) constitutes the basis for the Five-Colour Nutrition Label suggested in France to be put on the front-of-pack of food products. At the individual level, a dietary index (FSA-NPS DI) has been derived and validated and corresponds to a weighted mean of all FSA-NPS scores of foods usually consumed by the individual, reflecting the nutritional quality of his/her diet. Our aim was to investigate the association between the FSA-NPS DI and breast cancer risk in a large cohort.

Methods: This prospective study included 46,864 women aged over 35y from the NutriNet-Santé cohort (2009-2015) who completed at least three 24h dietary records during the first 2y of follow-up (median follow-up: 4.0y). 555 incident breast cancers were diagnosed. FSA-NPS DI was computed for each subject using the following nutrient content for 100g of each foods and beverages consumed: energy, total sugar, saturated fatty acid, sodium, fruits and vegetables (%), fibres and proteins. Higher values of the FSA-NPS DI correspond to a lower nutritional quality of the diet. Associations were characterized by multivariate Cox proportional hazards models.

Results: The FSA-NPS DI was directly associated with breast cancer risk ($HR_{1\text{-point increment}}=1.06$ (1.02-1.11), $P\text{-trend}=0.005$; $HR_{Q5vs.Q1}=1.52$ (1.11-2.08), $P\text{-trend}=0.002$). These associations were similar after the exclusion of cases diagnosed during the first year of follow-up.

Conclusions: In this prospective study, a higher FSA-NPS individual score was associated with an increased breast cancer risk. These results suggested that unhealthy food choices may be associated with a 52% increase in breast cancer risk (FSA-NPS DI ≥ 7.7 (Q5) vs. < 4.1 (Q1)), supporting the public health relevance of developing front-of-pack nutrition labels based on this score.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-13-02

Title: Long-term effects of weight loss on breast cancer biomarkers in postmenopausal women

McTiernan A, Duggan C, Tapsoba JdD, Mason C and Wang C-Y. Fred Hutchinson Cancer Research Center, Seattle, WA.

Body: Background: Obesity increases risk for postmenopausal breast cancer, and is associated with elevated blood levels of inflammation-related marker C-reactive protein (CRP), insulin, glucose, and the angiogenesis-related biomarker vascular endothelial growth factor (VEGF). The long-term effect of weight loss on these breast cancer risk biomarkers is unknown. This study aim was to test the 30-month effect of weight loss on breast cancer biomarkers in postmenopausal overweight/obese women, who had completed a randomized control trial test of behavioral weight loss and/or exercise compared to control.

Methods: From 1/2005-7/2008, women (N=438) were randomized to 1 of 4 arms: reduced calorie weight loss diet (D; N=118); moderate intensity aerobic exercise (E; 225 minutes/week) (N=117); both interventions (D+E; N=116); or control (C, no intervention; N=87). The D intervention goals were: 1200-2000 calories/day, dietary fat < 30% of calories, and $\geq 10\%$ loss of initial weight. The E goal was ≥ 45 minutes of moderate-intensity exercise 5 days/week. The D+E interventions were identical to those for D and E. A total of 157 women returned for a 30-month blood draw (18 months after end-of-study).

Analysis: We compared the average changes in outcomes from baseline to 30 months in weight loss (combining D and D+E groups) vs. no weight loss (combining C and E groups), according to the intention-to-treat principle, using generalized estimating equations (GEE). As a secondary, pre-planned analysis, we combined data for all women regardless of randomization group, and divided them into four categories based on change from baseline to 30 months: 1) gained weight or remained weight stable; 2) lost < 5% of baseline weight; 3) lost 5% - <10% of baseline weight; and 4) lost $\geq 10\%$ of baseline weight. We then compared 30-month analyte changes by these 30-month weight loss groups, with category 1 as referent.

Results: Mean 30-month weight changes from baseline were: D+E, - 7.7%; D, - 6.3%; E, -1.9%; and C, -3%. CRP was 22.5% lower than baseline for women randomized to a diet group, compared with a 4% reduction from baseline in women in a non-diet group ($p=0.07$). Insulin was 33% lower in women randomized to diet, while it was 20% lower in women not randomized to a diet group ($p=0.10$). Women who lost 5% - <10% of baseline weight at 30-months experienced an 18% ($p<0.001$) decrease in CRP compared to baseline, while those who lost $\geq 10\%$ had a 56% lower CRP level ($p<0.001$). VEGF decreased to a similar amount (14% -15%) in women who lost <10% of baseline weight; and decreased by 26% in those whose 30-month weight decreased by $\geq 10\%$ ($p<0.002$). Compared with baseline levels, insulin decreased by 16% in those who lost < 5% of baseline weight. In contrast, insulin levels decreased by 31% and 44% (each $p=0.004$), in those whose 30-month weight loss was 5% - <10% lower than baseline and $\geq 10\%$ lower than baseline, respectively. Glucose levels increased in women whose weight loss was < 5%, but remained stable in those whose 30-month weight was $\geq 5\%$ lower than baseline (each $p=0.04$).

Conclusions: Long-term weight loss of at least 5% may have biological effects relevant to breast cancer prevention in overweight or obese postmenopausal women.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-13-03

Title: Changes in the gut microbiome of post-menopausal women 2 weeks after initiating a structured weight loss intervention

Fabian CJ J, Kimler BF F, Umar S, Ahmed I, Befort CA A, Nydegger JL L, Kreutzjans AL L, Powers KR R, Klemp JR R, Spaeth KR R and Sullivan DK K. University of Kansas Medical Center, Kansas City, KS.

Body: Background

Change in the relative composition of the gut microbiome at the phyla level, particularly decreases in *Bacteroidetes* and increases in *Firmicutes* species, has been associated with both obesity and increased risk for breast cancer. It is unclear how rapidly the microbiome changes in response to a reduced calorie and fat diet during a weight loss intervention. As a planned sub-study of a clinical trial with a structured behavioral weight loss intervention with randomization to high dose omega-3 fatty acids or placebo (NCT02101970; clinicaltrials.gov), we evaluated changes in the gut microbiome after 2 weeks of dietary intervention.

Methods

46 post-menopausal women at increased risk for breast cancer with a BMI > 27 kg/m² had a baseline 3 day food record, DXA, and blood and breast tissue sampling for biomarkers. They were then started on a reduced fat and calorie diet (~1200 kcal/day from 2 portion-controlled entrees, 3 low calorie high protein shakes, and 5 servings of fruits/vegetables daily), recommendation to exercise 225 minutes per week, and a weekly behavioral intervention. Fecal samples were collected at baseline, after 2 weeks of diet but prior to study agent, and after 6 months of weight loss intervention. Stool samples were stored at -20°C until brought to the clinic, and then at -80°C until DNA extraction. Bacterial taxonomic classification was performed using real-time PCR and 16S pyrosequencing using specific 16S rRNA primers. Baseline Healthy Eating Index (HEI) was calculated from the 3 day food record; fruit and vegetable servings were obtained from weekly food logs.

Results

42 women completed the 6 month weight loss intervention. At baseline, median BMI was 31.0 kg/m² and HEI was 58 (range 28-90) with 12 and 23 servings of fruits and vegetables per week. Median relative weight loss at 6 months was -11.9 % (0 to -22.7 %). When dichotomized to relative losses of <10% vs >10% (which we have previously shown to be associated with significant improvement in blood and breast tissue risk biomarkers [Fabian Cancer Prev Res 2013]), women with 6 month >10% loss had favorable change in the two major stool phyla at 2 weeks with a median 10% increase for *Bacteroidetes* and 8% decrease for *Firmicutes*. Conversely, women with <10% loss showed a decrease (median -11%) in *Bacteroidetes* and an increase (median 16%) for *Firmicutes*. Fruit and vegetable consumption also differed between the weight loss groups. The >10% loss group had higher baseline consumption of vegetables and continued this after starting the diet. The more adherent a woman was to dietary recommendations in the first weeks of dietary intervention, the more likely she was to lose >10% weight by 6 months.

Conclusions

Favorable modulation of the gut microbiome early in a weight loss intervention is associated with subsequent substantial weight loss. Microbiome assessment after 6 months of weight loss intervention is in progress.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P4-13-04

Title: Efficacy of *dipsacus asperoides* (DA) in a model for triple negative breast cancer

Telang NT T, Nair HB B and Wong GYC YC. Palindrome Liaisons Consultants, Montvale, NJ; UT Health Science Center, San Antonio, TX and American Foundation for Chinese Medicine, New York, NY.

Body: Background: The triple negative breast cancer (TNBC) is characterized by the absence of estrogen receptor- α (ER- α), Progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2). This molecular subtype responds only to conventional cytotoxic chemotherapy and to small molecule based targeted therapy. These therapeutic options are associated with long-term systemic toxicity and acquired tumor resistance leading to compromised efficacy. These limitations emphasize identification of efficacious non-toxic agents for secondary prevention/therapy of TNBC. *Dipsacus asperoides* (DA) is a Chinese herb of a nutritional nature. The root of this herb represents an ingredient that is commonly included in the traditional Chinese herbal formulations for health management purposes. The present study examines the growth inhibitory effects of DA and identifies possible mechanistic leads for their efficacy in a preclinical cell culture model for TNBC.

Experimental Model, Herbal Extract and Biomarkers: The human mammary carcinoma derived ER- α ⁻, PR⁻ and HER-2⁻ MDA-MB-231 cell line represents the model for TNBC. Non-fractionated aqueous extract from DA represents the test agent. Anchorage dependent growth, anchorage independent (AI) colony formation, cell cycle progression and relevant pathway specific mechanistic assays represent the quantitative biomarkers for efficacy.

Results: Treatment of MDA-MB-231 cells with DA extract induces a dose dependent cytostatic growth arrest (IC₅₀: 0.0015%, IC₉₀: 0.0030%), and strongly inhibits AI colony formation. Within the cytostatic concentration range, DA treatment inhibits cell cycle progression via progressive G2/M arrest, suggesting abrogation of the G2/M checkpoint affecting DNA damage repair pathway. The induction of cellular apoptosis by DA is indicated by a dose dependent up-regulation of pro-apoptotic Caspase 3/7 activity.

Conclusion: These data identify potential mechanistic leads for the efficacy of DA in the present model. The present study validates a mechanism based approach to prioritize efficacious non-toxic herbal extracts for secondary prevention/therapy of the TNBC molecular subtype of clinical breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-14-01

Title: POWER-remote: A randomized study evaluating the effect of a remote-based weight loss program in women with early stage breast cancer

Santa-Maria CA A, Coughlin J, Blackford A, Carpenter A, Dalcin A, Huang C-Y, Lubner B, Schreyer C, Armanios M, Sharma D, Chaudhry M, Jerome GJ J, Snyder C, Appel L and Stearns V. Northwestern University; Johns Hopkins and Towson University Towson.

Body: Background: The majority of women diagnosed with breast cancer are overweight or obese, and gain weight after diagnosis. The Practice-based Opportunities for Weight Reduction (POWER) study reported that, in an obese population with cardiovascular risk factors, a scalable remote weight loss intervention with web support was equally effective to an in-person intervention (Appel NEJM 2011). We adapted the remote intervention for breast cancer survivors.

Methods: We conducted a phase II single-blind trial in which women with stage 0-III breast cancer and a BMI ≥ 25 were randomized to a remotely-delivered weight loss intervention with a study specific website (POWER-remote) or to self-directed weight loss. Participants were stratified by menopausal status and concomitant hormone therapy use. Weight was assessed at baseline, 6 and 12 months. The primary objective was to compare the proportion of women who lost $\geq 5\%$ of their baseline body weight after 6 months in the POWER-remote and the self-directed arms. A sample size of 80 patients yielded approximately 93.6% power to detect a difference in weight loss response of 19.0% in the self-directed arm and 38.2% in the POWER-remote arm with a one-sided type I error of 10%. We obtained blood samples for correlative studies including inflammatory biomarkers and assessment of telomere length at baseline and 6 months.

Results: From 2013-2015 we enrolled 96 women; 84 were evaluable for the primary analysis. Both cohorts had similar baseline characteristics including menopausal status, race (77% Caucasian and 20% African American in entire cohort), and BMI (average mean 32 kg/m²). The majority (93%) of patients received endocrine therapy, and 55% had completed chemotherapy. At 6 months 43.1% (95% CI 29.3–57.8) of women randomized to POWER-remote had lost $\geq 5\%$ of their baseline body weight, compared to 11.1% (95% CI 3.7–24.1) in the self-directed arm, $p < 0.001$. A significant difference continued at 12 months, and was observed in all subgroups (Table 1). Biomarker analysis will be presented at the meeting.

Table 1. Proportion of patients achieving $\geq 5\%$ weight loss after 6 and 12 months

	POWER-remote	POWER-remote	Self-Directed	Self-Directed		
	n	% [95% CI]	n	% [95% CI]	p-value	Interaction p-value for heterogeneity of treatment effect
Lost $\geq 5\%$ of baseline weight at 6 months						
All patients	43	43.1 [29.3, 57.8]	41	11.1 [3.7, 24.1]	<0.001	
Endocrine therapy	35	45 [29.3, 61.5]	32	11.1 [3.1, 26.1]	< 0.001	0.96
No endocrine therapy	8	36.4 [10.9, 69.2]	9	11.1 [0.3, 48.2]	0.11	
Chemotherapy	21	26.9 [11.6, 47.8]	25	3.7 [0.1, 19]	0.01	0.64
No chemotherapy	22	60 [38.7, 78.9]	16	22.2 [6.4, 47.6]	0.01	
Lost $\geq 5\%$ of baseline weight at 12 months						

All patients	30	31.4 [19.1, 45.9]	31	13.3 [5.1, 26.8]	0.006	
Endocrine therapy	26	32.5 [18.6, 49.1]	26	16.7 [6.4, 32.8]	0.04	0.99
No endocrine therapy	4	27.3 [6, 61]	5	0 [0, 33.6]	0.05	
Chemotherapy	11	19.2 [6.6, 39.4]	16	7.4 [0.9, 24.3]	0.07	0.72
No chemotherapy	19	44 [24.4, 65.1]	15	22.2 [6.4, 47.6]	0.07	

Conclusions: Sustained weight loss over 1 year is feasible in breast cancer survivors who undergo a remotely delivered weight loss intervention. Weight loss was observed irrespective of endocrine therapy or chemotherapy. These data will be used to design a new trial with a physical activity component.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-15-01

Title: Cost-effectiveness of pre-implantation genetic diagnosis for BRCA mutation carriers

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Body: Introduction: Management of *BRCA* mutation carriers is very expensive due to preventive surgeries and/or screening tests, as well as greater likelihood of cancer treatment. The related cancer burden and costs continue from generation to generation. One relatively new option for male or female *BRCA* mutation carriers, who wish to have children, is pre-implantation genetic diagnosis (PGD) of in vitro fertilized embryos. PGD eliminates the mutation from the descendants of these carriers. The purpose of this study was to model the cost-effectiveness of PGD.

Methods: We developed a Markov Model using TreeAge Pro 2016 and compared incidence of cancers, cancers-related death, costs, quality adjusted life-years (QALY), and incremental cost-effectiveness ratio (ICER) in the 2nd generation associated with conventional management of *BRCA* mutation carriers vs. PGD using a U.S. third-party payer's perspective with a lifetime horizon at a discount rate of 3% per year. In the model, health states were implemented to reflect the natural history of breast and ovarian cancer for women, and prostate cancer (and breast cancer in *BRCA2* mutation carriers) for men. Model data were obtained from published literature. Costs were determined from published data and insurance payment schedules.

Results: Our preliminary results show that for *BRCA1* mutation carriers, the PGD with IVF strategy is associated with an increase of 0.29 QALYs and costs an additional \$292.68 per person, translating to an ICER of \$1,014.25/QALY when compared with "No PGD", making it highly cost-effective. For *BRCA2* mutation carriers, the PGD with IVF strategy is associated with an increase of 0.17 QALYs and costs an additional \$4,916.88 per person, translating to an ICER of \$28,436.10/QALY when compared with "No PGD", making it cost-effective

Table 1

Strategy	Cost (\$)	Incr Cost (\$)	Eff (QALY)	Incr Eff (QALY)	Incr C/E (\$/QALY)
BRCA 1					
No PGD	323,347.22		28.41		
PGD	323,639.89	292.68	28.7	0.29	1,014.25
BRCA 2					
No PGD	318,723.02		28.52		
PGD	323,639.89	4,916.88	28.7	0.17	28,436.10

Conclusion: PGD for both *BRCA1* and *BRCA2* mutation carriers reduces cancer burden, increases QALYs and, is very cost-effective in the 2nd generation cohort, making this an attractive option from the perspectives of patients and public payers. Our estimates are conservative because the cost-effectiveness of PGD will likely improve further if subsequent generations are included in the model, given the expected further reduction in cancer burden and associated cost-savings in subsequent generations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-15-02

Title: Investigating the use estrogen receptor β agonists in the prevention of breast cancer using a transgenic mouse model

Samayoa C, Krishnegowda NK K, Vadlamudi RK K and Tekmal RR R. University of Texas Health Science Center at San Antonio, San Antonio, TX.

Body: Breast cancer is the most common cancer among women worldwide. In the United States, 1 in 8 women will develop breast cancer during her lifetime, and in 2016 over 240,000 new cases will be diagnosed. Incidence rates differ by geographic location, with women living in Asia having the lowest rates. However, after migrating to the U.S and adopting a western diet their rates increase. This highlights the importance of lifestyle, including diet, in modulating breast cancer risk. Soy has been previously implicated as the dietary component contributing to the reduced breast cancer rates in Asian women. Compounds that can selectively activate Estrogen Receptor β have been identified in plants, including soy. Given the tumor-suppressive properties of ER β , it may be possible to use these agonists in the chemoprevention of breast cancer.

The objective of this study was to investigate the utility of using ER β agonists in the prevention of breast cancer using a transgenic mouse model. MMTV-HER2/neu mice develop premalignant lesions at 4-5 months, and tumors starting at month 7 due to overexpression of the Her2/neu proto-oncogene. MMTV-HER2/neu mice were treated with 2 different ER β agonists, S-equol or LY500307, for 3 months and evaluated for branching, hyperplasia and differential gene expression. When compared to controls, ER β agonist-treated mice exhibited a significant decrease in branching and ductal hyperplasia, with no change in body weight. Differential gene expression analysis revealed 218 modified genes in response to S-equol treatment, and 258 genes modified by LY500307 treatment, with an overlap of 36 genes. Pathway analysis identified an enrichment for chemokines signaling pathways, particularly TNF, in the reversal of hyperbranching resulting from treatment with ER β agonists. Although previous studies have demonstrated crosstalk between ER's non-genomic signaling and growth factor signal transductions pathway, this is the first study to demonstrate the impact of ER β activation on HER2/neu mediated pre-neoplastic changes. Our study suggest that ER β agonist treatment may be a valuable therapeutic option for the chemoprevention of breast cancer in women at increased risk.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-15-03

Title: Regulation of miRNA-29c and its gene targets in preneoplastic progression of triple negative breast cancer

Bhardwaj A, Tachibana K, Ganesan N, Rajapakshe K, Singh H, Gunaratne P, Coarfa C and Bedrosian I. UT M.D. Anderson Cancer Center, Houston, TX; Baylor College of Medicine, Houston, TX and University of Houston, Houston, TX.

Body: Introduction: Little is understood about the *early* molecular drivers of the triple negative breast cancer making identification of women at risk and development of targeted therapy for prevention a significant challenge. **Methods:** Here, by deep sequencing of TNBC- cell line based breast cancer progression system we have identified miRNA-29c and its functional gene targets to be potentially involved in the normal to preneoplastic transition during TNBC progression. We have used cell line based functional assays that are relevant in early tumorigenesis such cell proliferation (ki67), and colony formation assay to study the growth inhibitory potential of these miRNA and their gene targets. To identify direct gene targets of miRNA-29c, we cloned the 3'untranslated region containing miRNA-29c binding sites from predicted gene targets in a luciferase reporter vector, pmiRGLO and studied the potential of miRNA-29c overexpression on the repression of luciferase reporter activity indicating their direct gene regulation. **Results:** Our deep sequencing results and their further validation by QPCR revealed miRNA-29c to be lost during the TNBC progression, and its forced expression to inhibit cell proliferation and colony formation of preneoplastic (MCF10AT1) and ductal carcinoma in situ (MCF10DCIS) cells. We found miRNA-29c to directly bind in 3'UTR of TGIF2, CREB5, AKT3 and CDK6 and regulate their expression as shown by our luciferase assays. We also found miRNA-29c binding to 3'UTR of these gene targets to be functionally relevant as TGIF2, CREB5 and AKT3 were able to rescue the inhibition in cell proliferation and colony formation assay caused by loss of miRNA-29c in preneoplastic cells. Further confirming the relevance of these miRNA-29c gene targets and pathways in TNBC tumorigenesis, inhibition of PI3K, which is upstream of AKT3, inhibits cell proliferation in MCF10AT1 and DCIS cells. We also examined the regulation of tumor suppressor miRNA-29c to study the mechanisms responsible for its loss during breast cancer development. We found c-myc and EZH2 driven epigenetic mechanism as well as DNA methylation in part to cause the loss of miRNA-29c during TNBC progression. Consistently, we found a pan HDAC inhibitor and a DNA methylation inhibitor to relieve the suppression of miRNA-29c. **Conclusions:** Together, these results indicate that loss of miRNA-29c plays a central role in preneoplastic development of breast cancer and efforts directed at inhibition of its target pathways or rescue of miRNA-29c itself may provide novel opportunities for prevention of TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-15-04

Title: Molecular mediators of mammographic density

Ironside A, Hawkesford K, Gomm J, Haywood L, Goulding I, Wang J, Lamaziere A, Poirot M, Silvente-Poirot S, Chelala C and Jones JL. Centre for Tumour Biology, Barts Cancer Institute, London, United Kingdom; Centre for Molecular Oncology, Barts Cancer Institute, London, United Kingdom; Sterol Metabolism and Therapeutic Innovations, Cancer Research Center of Toulouse, INSERM UMR 1037, Toulouse, France and Sorbonne Universités-Université Pierre et Marie Curie-Paris 6, Paris, France.

Body: Introduction

Mammographic density (MD), created predominantly by increased stromal tissue, is a major risk factor for breast cancer, though little is known about the biological mechanisms mediating it.

Tamoxifen prevents breast cancer in a subset of high-risk women via mechanisms that appear dependent on reduction of MD. Animal models suggest tamoxifen remodels the mammary stroma to a tumour-inhibitory phenotype. This study aims to analyse the effect of tamoxifen on human breast fibroblast function and identify pro-tumourigenic pathways contributing to the density-associated risk.

Methods

Primary human breast fibroblasts from normal, high risk or breast cancer patients were treated with hydroxytamoxifen (100nM-5uM), the active metabolite of tamoxifen. Fibroblast function was analysed by measuring: proliferation, expression of stromal proteins fibronectin and collagen 1; effects on TGF- β signalling via SMAD phosphorylation and upregulation of the myofibroblast marker SMA. Genome wide analysis was performed using RNA-Seq. Significantly deregulated pathways were validated by quantitative real time PCR, western blotting and mass spectrometry.

Results

Fibroblasts from 25 patients were treated with hydroxytamoxifen. All patients showed reduced proliferation with treatment. 62% of patients showed reduced fibronectin expression. Collagen 1 expression and TGF- β -mediated upregulation of SMA and fibronectin were consistently inhibited by tamoxifen.

RNA-Seq analysis revealed downregulation of Wnt signalling, an established pro-fibrogenic and pro-tumourigenic pathway, and other stromal signalling pathways including matrix/receptor interaction, focal adhesion signalling and collagen formation.

Intriguingly, there was significant modulation of many metabolic pathways, including components of the microsomal anti-oestrogen binding site (AEBS).

Binding of tamoxifen to the AEBS inhibits cholesterol epoxide hydrolase (ChEH) enzyme activity, promoting an anti-tumourigenic phenotype. The effects of tamoxifen on fibroblasts could be replicated using tesmilifene, a commercially available inhibitor of ChEH. Mass spectrometry analysis confirmed an altered cholesterol metabolite profile in the tamoxifen treated fibroblasts.

Conclusion

These data indicate that tamoxifen can directly remodel the mammary stromal microenvironment, generating a less 'reactive' stroma. Thus, tamoxifen impacts on multiple pathways, many independent of the oestrogen receptor, to create a tumour-inhibitory microenvironment. This offers exciting potential for patient monitoring and alternative breast cancer prevention strategies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-16-01

Title: Evaluation of miracle mouthwash (MMW) plus hydrocortisone or prednisolone mouth rinses as prophylaxis for everolimus-associated stomatitis: Results of a randomized phase II study

Jones VE E, McIntyre KJ J, Paul D, Wilks ST T, Ondreyco SM M, Sedlacek SM M, Melnyk Jr. AM M, Oommen SP P, Wang Y and O'Shaughnessy JA A. US Oncology Research, Inc., The Woodlands, TX; Yakima Valley Memorial Hospital/North Star Lodge, Yakima, WA; Texas Oncology, Dallas, TX; Rocky Mountain Cancer Centers, Denver, CO; Texas Oncology, San Antonio, TX; Arizona Oncology, Glendale, AZ; Texas Oncology, Abilene, TX; Texas Oncology, Fort Worth, TX; McKesson Specialty Health, Inc., The Woodlands, TX and Texas Oncology/Baylor-Charles A. Sammons Cancer Center, Dallas, TX.

Body: Background: Oral stomatitis is a frequent adverse event (AE) associated with mTOR-inhibitor therapy, and can impact adherence. In BOLERO-2, patients (pts) with hormone receptor-positive (HR+) metastatic breast cancer (MBC) treated with exemestane plus everolimus (EVE), the incidence of all-grade (G) stomatitis or related AEs was 67%, with 24%/8% of pts developing G2/G3 stomatitis or related AEs, respectively (Perez et al ASCO 2013 Abs 7029). In BOLERO-2, 24% of pts required EVE dose reduction for stomatitis (Rugo et al Ann Oncol 2014;25:808). This study evaluated 2 steroid-based mouth rinses for the prevention or amelioration of oral stomatitis in pts with MBC treated with EVE. **Methods:** This prospective randomized phase II study enrolled postmenopausal pts (planned accrual=100) with HR+ MBC within the US Oncology Network who were initiating therapy with an aromatase inhibitor plus EVE (10 mg/day)(AIE). Pts were randomized 1:1 to prophylactic therapy with 1 of 2 oral rinses (ARM 1: MMW 480 ml recipe: 320 mL oral Benadryl, 2 g Tetracycline, 80 mg Hydrocortisone, 40 mL Nystatin suspension, water; or ARM 2: Prednisolone (P) 15mg/5mL oral solution, 1.8% alcohol). Pts were instructed to swish/expectorate 10 ml of the assigned rinse 4x daily starting with D1 of EVE treatment, for a total of 12 wks. The primary objective was to determine the incidence of G \geq 2 stomatitis or related oral AEs during the first 12 wks of treatment. Based on a historical estimate that \geq 37% of pts receiving AIE develop G \geq 2 stomatitis, 50 pts for each arm were required to detect a reduction of the incidence of G \geq 2 stomatitis from 37% to \leq 20%, with alpha = 0.05, 80% power, and a 1-sided test. Secondary objectives included assessment of AEs (all grades), determination of the percentage of pts requiring dose interruption/reduction of EVE or discontinuation of therapy due to toxicity, and evaluation of the impact of the oral rinses on the duration and severity of stomatitis. **Results:** As of 5/30/2016, a total of 104 pts have been randomized and 100 pts have received treatment (49 MMW; 51 P). Median age was 61 yrs (range 31-82 yrs). The incidence of stomatitis and related oral AEs (any grade) during the first 12 wks was 29% (n=14/49) and 27.5% (14/51) in the MMW and P arms respectively. The incidence of G2 oral AEs was 12% (6/49) and 8% (4/51) with MMW and P respectively. There was only 1 G3 oral AE (MMW arm), and no G4 events. There was 1 EVE dose reduction (MMW) and 4 EVE dose delays (3 MMW, 1 P) during the first 12 wks of treatment. No pts stopped the steroid mouth rinse therapy due to mouth rinse-related toxicity. **Conclusion:** These prospective data provide evidence of a reduced incidence of mTOR-associated oral AEs with prophylactic use of a steroid mouth rinse. The 29%/27.5% incidence of all-grade and 12%/8% incidence of G2 oral AEs, with only 1 G3 event, compare favorably with the 67% and 24%/8% incidence of all-grade and G2/3 stomatitis, respectively, in BOLERO-2. These data also show the safety and tolerability of these 2 steroid mouth rinses. Prophylactic use of steroid mouth rinses substantially decreases the incidence of G2/3 stomatitis and the need for EVE dose modifications.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-16-02

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-17-01

Title: YSC's "shady pink elephant" end of life series

Esser MR R, Rowe J, Schapmire T and Lewis S. Young Survival Coalition, New York, NY and University of Louisville, Louisville, KY.

Body: Background

Young Survival Coalition (YSC) is the premier organization dedicated to the critical issues confronted by young women diagnosed with breast cancer. Young women diagnosed with metastatic breast cancer (YWMBC) in particular, face unique concerns. As a result of a large survey to its metastatic constituency from September 2013 through February 2014, YSC gained important information about the lack of knowledge and education when it comes to end of life (EOL) planning and decisions. The survey was a result of YSC's Research Think Tank, which included a metastasis work group that identified the psychosocial needs of YWMBC as a priority.

Based on the results of this survey which showed 71% of YWMBC had not made EOL plans, YSC decided that offering education and tools regarding EOL was an important intervention to offer. In doing so, young women affected by breast cancer, young adult cancer survivors, co-survivors and healthcare providers will be better prepared to have difficult conversations by allowing stigma to be removed, intentions to be shared, and plans to be made, all to honor the young woman's wishes.

Methods

YSC created a three part end-of-life series called the "Shady Pink Elephant." (hereinafter "Series") Each part featured a subject matter expert and was offered as a Facebook event where the speaker was live-streamed and recorded. YSC also offered free continuing education credits for nurses and social workers. The recorded events are stored on YSC's YouTube channel as enduring material. The three parts include: (a) The Research and Benefit of Introducing Palliative Care Early; (b) Let's Have Dinner and Talk about Death; and (c) The "Nuts and Bolts" of End of Life Planning. YSC collaborated with the University of Louisville and received IRB approval to study the entire series as intervention. The study is in process and will conclude later in 2016.

Results

Nine participated in Series Part One of the live event. For Series Part Two, 31 participated in the live event. In the third and final part, 33 participated in the live event. The three parts housed on YSC's YouTube channel have been viewed a total of 747 times to date: 411 views for part one, 156 views for part two and 180 for part three. Thirty-three sought continuing education credit. Fourteen social workers and 5 nurses contacted the approving organization to receive continuing education credit for Series Part One, and 10 social workers and 1 nurse contacted the approving organization to receive continuing education credit for Series Part Three.

Conclusions

Live-streamed, recorded Facebook events are a novel and forward-thinking mode of programmatic offering. YSC learned through this process that live-streamed events are a feasible way to offer programming that reaches a larger and broader audience of young women affected by breast cancer and their loved ones.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-17-02

Title: Design, implementation and evaluation of a smoking cessation intervention for patients undergoing breast cancer surgery

Nolan M, Ghosh K and Warner DO O. Mayo Clinic, Rochester, MN.

Body: Introduction:

Smoking is a risk factor for poor outcomes following breast reconstructive surgery, including wound dehiscence and infection.

Women who would choose to have reconstructive surgery after mastectomy for breast cancer are unable to do so if they have not quit smoking. The specific aims of this project are to 1) design and implement an intervention to assist these patients in quitting and 2) evaluate its efficacy.

Methods:

Baseline data regarding current practices was collected via a chart review of 75 smoking breast cancer surgery patients from 1/1/2012-12/31/13. Charts were analyzed for documentation of smoking status, quit advice, resources provided and used, and smoking status at follow-up. Formative research informed the design of an Ask-Advise-Refer intervention for all new breast cancer patients seen at Mayo Clinic.

Intervention Design:

Smoking patients were identified prior to their initial Breast Cancer evaluation at Mayo Clinic, Rochester, during a standard intake call. Smoking status was documented by the rooming assistant to alert the physicians of smoking status, and physicians asked these patients about their smoking behavior, discussed the risks of smoking both for surgical procedures and breast cancer, and offered a Nicotine Dependence Consultation with a tobacco treatment specialist in an "opt-out" strategy that emphasized the importance of smoking cessation as part of the breast cancer treatment process. The advice and referral was documented in the medical record. Evaluation of the intervention was performed via a chart review of patients seen after one year of implementation.

Intervention Evaluation Results:

Breast clinic physicians documented smoking status in all patients seen after implementation. Referral to the Nicotine Dependence Center for cessation counseling increased from 29% (22/75) to 74% (20/27). Among those referred, attendance at the consultation increased from 41% (9/22) to 75% (15/20), suggesting stronger provider encouragement of cessation services. 30-day abstinence rates for those who attended a consult prior to 5/1/16 were 46% (6/13), which is consistent with baseline data.

Conclusions:

An Ask-Advise-Refer intervention for new breast cancer patients seen at Mayo Clinic, Rochester, who report current smoking, has been associated with higher rates of provider counseling on tobacco dependence, referral to available services, and patient attendance at referrals. Attending a referral greatly increases a patient's chances of quitting smoking for at least 30 days compared to average spontaneous quit rates in this population. This model is low-cost, time-efficient for providers, and applicable to most practice settings. Finding ways to make this intervention self-sustaining and applicable to multiple other practices could greatly improve our patient's health outcomes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-17-03

Title: Global Cancer Institute online tumor boards to improve global patterns of clinical practice for breast cancer

St. Louis J, Bukowski A, Paulino E, Ferreyra ME E, Nunes J, Mejia G, Duarte C, Ruiz R, Touya D, Polo S, Chavarri-Guerra Y, Moreno J, Georgieva N, Tsolko T, Obayedullah Baki M, Luna HC C and Goss PE E. The Global Cancer Institute, Boston, MA; Massachusetts General Hospital Cancer Center, Boston, MA; Instituto Nacional do Câncer, Rio de Janeiro, RJ, Brazil; Harvard Medical School, Boston, MA; Marie Curie Hospital, Buenos Aires, Argentina; Hospital Erasto Gaertner, Curitiba, Brazil; Hospital Clinico Viedma, Cochabamba, Bolivia; Institution Nacional de Cancerologia, Bogota, Colombia; Instituto Nacional de Enfermedades Neoplásicas, Lima, Peru; Hospital de Clinicas, Montevideo, Uruguay; INCAN, Guatemala City, Guatemala; Instituto Nacional de Ciencias y Nutrition Salvador Zubiran, Mexico D.F., Mexico; Instituto Oncologico Nacional, Panama City, Panama; MHAT Nadezhda, Sofia, Bulgaria; Lviv State Oncological Regional Centre, Lviv, Ukraine; Obayedullah-Ferdousi Cancer Foundation, Dhaka, Bangladesh and National Kidney and Transplant Institute, Quezon City, Manila, Philippines.

Body: Background: The Global Cancer Institute (GCI) breast cancer multi-disciplinary tumor boards (MTBs) are live, online telemedicine discussions of breast cancer patient case scenarios between breast cancer specialists in low- and middle-income countries (LMICs) and expert breast cancer specialists in the United States (US). In the US MTBs are routinely held in most cancer centers and have been shown to improve patient outcomes and patient and family quality of life. GCI launched breast cancer MTBs in 2012 with the goals to improve breast cancer patient care in underserved populations globally, to establish an online platform to allow live communication and collaboration among oncologists, and to serve as an educational tool for oncologists.

Methods: During our MTBs case scenarios are presented by global oncologists for discussion and input by a panel of both community/tertiary care expert breast oncologists from our global network. During each MTB, three cancer centers present challenging breast cancer patient scenarios. Patient scenarios are presented in English, according to a standard PowerPoint template. After presentation guideline - or clinical trial-based discussions are held for each case. As the patient cases originate from oncologists in LMICs, optimal and best locally available clinical care in rural and remote settings are discussed. For educational purposes the MTBs and the associated YouTube panel discussions are archived online and can subsequently be viewed by practicing oncologists and trainees globally. Links to relevant international guidelines, published and ongoing clinical trials, and other educational resources are also provided to all MTB attendees.

Results: Since its initiation in 2012, the GCI MTBs have engaged a network of 370 oncologists in LMICs and 20 expert panelists from nine cancer centers in the United States. Together the oncologists in LMICs represent 28 tertiary cancer centers and 116 community oncologists in 19 countries across Latin America, Eastern Europe, Asia, and Africa.

Conclusions: GCI breast cancer MTBs are a powerful educational and networking tool for oncologists in LMICs to improve their patterns of clinical practice, conduct multi-disciplinary discussions and access research collaborations. GCI invites oncologists throughout Latin America, Europe, Asia, and Africa to join our tumor boards and further expansion of its MTB network. GCI currently surveys oncologists in our network before and after attendance of MTBs to measure modifications in oncologists' practice and adherence to international clinical practice guidelines.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-17-04

Title: Results of the continuing education program in oncoplasty and breast reconstruction of the Brazilian Society of Mastology at Araujo Jorge Hospital in Goiania

Paulinelli RR R, Filho JWCM C M, Ribeiro LFJ J, Freitas-Junior R and Urban CA A. Hospital Araujo Jorge, ACCG, Goiania, GO, Brazil; Federal University of Goias, Goiania, GO, Brazil; Positivo University, Curitiba, PR, Brazil and Hospital Nossa Senhora das Graças, Curitiba, PR, Brazil.

Body: Oncoplastic and reconstructive surgery is an important part of the treatment of breast cancer. In Brazil there is a specialization in Mastology since 1978, fully dedicated to manage all diseases of the breast. There are 2 years of specific residence training, after specialization in General Surgery or Gynecology. Incorporation of the reconstructive surgery is not uniform among all Breast Units around Brazil. Therefore, the Brazilian Society of Mastology has created an official program of continuing education. **Objective:** To evaluate the effectiveness of a Hands On Educational Program in Oncoplasty and Breast Reconstruction of the Brazilian Society of Mastology at the Hospital Araújo Jorge, in Goiânia, in 2015. **Materials and Methods:** Twelve mastologists were selected as trainees, based on the curriculum score. The program was divided into 10 monthly modules of two days (24h), with 25% of theory and a 75% of surgery. Activities were coordinated by two local and two invited teachers. The effectiveness was measured via an on-line questionnaire. To measure the improvement in medical practice it was used the modified classification of Urban and Lebovic, for the complexity of the reconstructive procedures. They were considered as class 1: monolateral partial reconstructions, esthetic incisions, periareolar de-epidermization, local reshaping; class 2: bilateral procedures, nipple-areolar reconstruction, lipofilling, breast augmentation, mastopexy; class 3: reconstruction with expanders or implants; class 4: myocutaneous flaps. Statistical analysis was performed by SPSS. It was significant a $p < 0.05$. Results: Twelve mastologists participated, with a mean age of 42.92 (± 10.27). They performed 213 procedures, in 88 patients, using the main techniques for breast reconstruction. All students declared themselves satisfied with the course and they would indicate it to others. At the beginning, most students had some experience in local flaps. Half of the students referred trouble in finding other colleagues that could perform the reconstructions. At the end, most students declared themselves comfortable to perform partial or total reconstruction of the breast with local flaps, mammoplasties, fat grafting, expanders and implants. Most of them were comfortable to resolve their own complications. More than a third were safe in performing miocutaneous flaps. All mastologists said to be interested in continuing technical improvement in different ways.

Table 1. Type of procedures usually executed by the trainees according to the modified Urban classification, before and after the course.

	Before the course	After the course	p
Class 1	8 (80%)	10 (83%)	<0.01
Class 2	1 (10%)	9 (75%)	
Class 3	1 (10%)	12 (100%)	
Class 4	1 (10%)	5 (42%)	
Cosmetic breast reductions or augmentation	0 (0%)	3 (25%)	

Table 2. Proportion of oncoplastic and reconstructive procedures executed by trainees, in clinical practice, before and after the course.

	Before	After	p
None	4 (36%)	0 (0%)	<0.01

<25%	4 (36%)	0 (0%)	
26 - 50%	2 (18%)	2 (18%)	
51 - 75%	0 (0%)	3 (27%)	
>75%	0 (0%)	4 (36%)	
100%	1 (9%)	2 (18%)	

Conclusion: The current program in Goiania benefited many patients and brought a great contribution to the training of the participants.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-17-05

Title: A school-based breast health (BH) educational program to increase breast cancer awareness in a rural Mexican community: A qualitative comparative analysis of students', relatives' and teachers' perceptions

Soto-Perez-de-Celis E, Rojo-Castillo MP and Chavarri-Guerra Y. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Tlalpan, Mexico City, Mexico and Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Tlalpan, Mexico City, Mexico.

Body: Background: In order to increase breast cancer awareness, we implemented a school-based educational program for female adolescents in a rural Mexican community, aimed at increasing knowledge and promoting intergenerational transmission of information to their female relatives. Here we report the perspective of the students, their female relatives and the school teachers on the program's impact on the adolescents and their community, as well as the major emergent themes related its implementation.

Methods: Adolescents enrolled in a public rural Mexican middle school were invited to participate in the program. They received age-appropriate, culturally sensitive sessions from expert educators focused on transmitting BH knowledge in their household. The students' opinions of the program were evaluated using open ended questions immediately after its completion. The opinion of the students' female family members and of the school teachers were recorded four months after the intervention using open ended surveys and semi-structured interviews. The interviews were conducted at the school by two oncologists who designed and implemented the program and were recorded in video and audio formats. Data was coded using a narrative research approach, and a constructivist paradigm was used to explore emerging themes. NVivo software was used for qualitative data analysis.

Results: The surveys were answered by 126 students, 185 family members and 18 teachers. Seven teachers then participated in semi-structured interviews. The following major themes were identified as related with the BH educational program by students: (1) the importance of breast self-examination; (2) the power obtained through knowledge and learning; and (3) the importance of disseminating the obtained information. The following major themes were identified by female relatives: (1) the importance of disease prevention; (2) knowledge to perform self-examination and seek medical attention and (3) empowerment through information. An analysis of the teachers' opinions identified the following major themes: (1) the program aided in discussing intimate topics; (2) the relevance of disseminating information in the community; (3) training other teachers and survivors for implementation; and (4) the importance of the acquisition of new knowledge. The relevance of obtaining and disseminating new knowledge was a common emerging theme among the three groups. Interestingly, both the students and their relatives highlighted the power of knowledge in empowering women and allowing them to prevent diseases and take care of their own health.

Conclusions: Obtaining feedback from the relevant stakeholders and promoting active participation of the community is essential for the implementation of successful school-based education programs in cancer. We identified relevant themes related to BH education which could be useful for designing and implementing future programs. Providing adolescents from underserved populations with new knowledge and tools to take care of their health can empower them and aid them not only in preventing disease but in disseminating that knowledge in their communities.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-17-06

Title: First posts: A content analysis of an online breast cancer community user's initial postings

Marmor RA A, Ward EP P, Epstein E, Reghunathan M, Schoenbrunner A, Unkart JT T, Blair SL L and Huh J. University of California San Diego, San Diego, CA.

Body: Introduction: Online breast cancer communities provide users with both information and emotional support. Members of these communities include both patients and caregivers. We aimed to characterize the differences in content between patients' and caregivers' first posts.

Methods: 20189 posts were downloaded from a public online breast cancer community. Posts were sorted by user name and date to identify unique posters and coded for user demographics and content. Descriptive statistics (e.g. chi-square test) characterized differences between patients and caregivers in terms of topics and content.

Results: Of the 1827 unique posters, 83% were identified as the patient (n=1277, 70%) and 13% were identified as a caregiver (n=230). 835 (69%) patients had been previously diagnosed with breast cancer. Chemotherapy was the most common topic among cancer patients

Breast Cancer Patients' Topics of Conversation

Topic	N(%)	Explanation	Example
Chemotherapy	296 (36)	Patients discussing the decision for chemotherapy, or their experiences with it.	"I was allergic to some of the chemo meds"
Radiation	255 (31)	Patients discussing the decision for radiation, or their experiences with it.	"I wanted to answer your query about radiation..."
Estrogen Modulating Therapy	156 (19)	Patients discussing the decision to take estrogen modulating medications (e.g. Tamoxifen) or their experiences with it.	"I don't know if I'm going to take this with all the side effects I read."
Surgery	55 (7)	Patients discussing surgical options for breast cancer treatment (not reconstruction) or experiences with the procedures.	"I don't want them to leave half of my breast with a chance for a new diagnosis"
Reconstruction	49 (6)	Patients discussing reconstruction options or experiences with the procedures.	"I recently had a TE recon and it went fine but a week later i developed an infection..."

. Patients tended to seek information about physical concerns more often than psychosocial concerns

Thematic Content of Posts: Patients v. Caregivers

Domain	Patients n(%)	Caregivers n(%)	Chi-Square p-value	Explanation	Example
Discussing Physical Issues	234 (29)	25 (11)	<.001	Discussing physical symptoms as a result of cancer or treatment.	"I was allergic to some of the chemo meds"
Seeking Emotional Support	113 (14)	34 (15)	<.001	Poster discusses feeling upset, or directly asks for support.	"Thanks for listening to my pity party..."
Discussing Psychosocial	89 (11)	21 (9)	.165	Poster mentions psychological issues including depression or anxiety, or with	"I also found help through the use of an antidepressant...I

Issues				social sequelae of cancer such as marital difficulties.	feel like a fog has lifted.”
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. In contrast, caregivers used the community significantly more often as a source of emotional support ($p < .001$), although they also sought information on how to best support patients.

Conclusion: First post content varied depending on if the poster was a patient or a caregiver. Patients' first posts were often information-seeking about physical concerns, whereas caregivers tended to use the forum as a source of emotional support. By analyzing the frequency of topics and content of posts, clinicians may better understand the educational needs of patients and caregivers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-18-01

Title: Waiting for Treatment: Addressing Inequities in access to essential medications for metastatic breast cancer

Faucette C. Canadian Breast Cancer Network (CBCN), Ottawa, ON, Canada.

Body: Introduction:

Of the women in Canada diagnosed with breast cancer each year, 5% will have an initial diagnosis of metastatic breast cancer and many more will recur with metastatic disease. Despite global advancements in metastatic breast cancer, access to new drugs in Canada remains inequitable.

Objective:

To examine the present situation in Canada for drug approval and decision-making by provinces and territories around new metastatic drugs and to assess processes in the system causing the greatest lags, the range in wait times for new treatments by province and the jurisdictions most affected by wait time delays.

Methodology:

- A bilingual survey in spring 2015, focusing on access to metastatic treatments across Canada. 98 women responded to the survey from all Canadian jurisdictions except Yukon.

-A case study of 4 metastatic therapies undergoing multi-step drug review.

Results:

Systemic Delays:

Patients endure 1-2 years for drugs to be approved for sale in Canada and further delays in access through the pCODR, pCPA and provincial drug assessment processes. The longest delays occur at the provincial review stage.

Inequitable Access to Treatments across Provinces:

Lack of firm deadlines for the provinces to list a drug on their formulary once a drug has been approved pricing negotiated. Often a 2-year time lag or longer between provinces in making reimbursement decisions, resulting in inequitable access across the country.

Line Sequencing Access Delays:

Further limitations on access created by the restrictions that provinces place on when drugs can be used in the course of treatment. Formularies often cover older drugs that are less costly, and as such it is a challenge to secure coverage of newer therapies.

Conclusion:

Wait time delays create a significant barrier to patients receiving optimal treatment and care. Metastatic patients are forced to endure wait time delays of 2-4 years before accessing new treatments. These delays occur at nearly every stage of the approval process, with the greatest waits occurring at the provincial level.

Lags in treatment access across the country also have an impact on standards of care. Patients in provinces with timely approvals, have access to more treatment options than those in provinces where patients must wait for new treatments to be added to the formulary.

Lengthy wait times for new treatments complicate a patient's prognosis. Slowing the progression of their disease and maintaining a better quality of life is of critical concern and metastatic patients with urgent treatment needs cannot wait for new treatments to become publicly accessible. For these patients, expedited access to a diversity of treatment options can make all the difference in ensuring optimal health outcomes and improved quality of life.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-18-02

Title: Peer navigation for people affected by hereditary breast, ovarian and related cancers: Results from the first six months

Rezende LF F, Cohen S, Rose D and Friedman SJ J. FORCE - Facing Our Risk of Cancer Empowered, Tampa, FL.

Body: People affected by hereditary breast, ovarian, and related cancers (HBOC) due to a mutation in BRCA, or another gene that increases cancer risk, have unique support and information needs and face medical decisions that differ from survivors of sporadic breast cancer and from people at average risk for these cancers. Finding peers to talk to can be challenging, especially for people living outside of metropolitan areas.

FORCE (Facing Our Risk of Cancer Empowered) developed our Peer Navigation Program to provide personalized, expert-reviewed resources and 1:1 support for people affected by HBOC, including breast cancer survivors and people at high risk for breast cancer due to a mutation in BRCA or another gene that increases cancer risk.

The program utilizes a custom database that matches individuals seeking support with trained Peer Navigators who have faced similar medical challenges and decisions. Users complete an intake form, providing basic demographic, medical, and geographic information in order to match them as closely as possible to a peer volunteer. The user selects from 21 specific topics of interest to them. This generates a personalized, expert-reviewed resource guide on each selected topics, which individuals can use to make informed, shared decisions with their healthcare providers.

Trained Peer Navigators conduct one-hour phone calls with program users to discuss the personalized resources and provide non-judgmental emotional support. Our goal is to match users and complete the navigation process within one week from the time they submitted their intake form. After a navigation session, both the Peer Navigator and the program participant are asked to fill out an evaluation.

All Peer Navigator volunteers undergo a written and telephone interview to assure their readiness to help others. Once they pass this screening process, they complete a series of online VolunteerFORCE Academy training webinars and complete a profile form providing information about their demographics, personal and medical situation, and medical decisions.

Volunteers receive training on the following: FORCE 101, HBOC 101, Active Listening Skills and Peer Navigation instructions. All webinars have been reviewed and approved by a member of FORCE's Scientific Advisory Board and our Vice President of Education. The webinars stress several important themes, including: encouraging users to discuss information with medical experts, avoiding dispensing of personal or medical advice, and maintaining a non-judgmental perspective.

We have 104 trained peer navigators, both male and female, ranging in age from 21 – 73. Both cancer survivors, as well as high-risk individuals are represented. Peer volunteers come from diverse backgrounds and geographic locations, and have made a variety of medical decisions about genetic testing, cancer screening, chemoprevention, and risk-reducing surgeries.

The program launched in April 2016. We will present results from post-call evaluation surveys completed from April 1, 2016 through September 30, 2016. Data on most commonly requested topics, user satisfaction, ease of use, and intention to use the information in shared decision-making with their health care provider will be presented.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-18-03

Title: Measuring what is important to patients in clinical trials: Hearing the patient's voice

Spears PA A, Devine P and Finestone S. Cancer Information & Support Network, Auburn, CA.

Body: Patients who participate in clinical trials are important to clinical research and contribute to the advancement of medical discoveries. However, typical clinical trials do not always capture information that is important to patients: how they feel, function and survive. The only way to accurately measure how a patient feels or functions is to ask them. This can be done through the administration of health related questionnaires that are filled out by the patient without interpretation from anyone else; these are referred to as Patient Reported Outcomes (PROs). Although, PRO assessments have been used in clinical trials for the past 30 years through the collection of global quality of life (QOL) and later health related QOL (HR-QOL) measurements, the information has been too broad and has not been precise enough to use in drug approvals, descriptive drug label information or to inform other patients.

There is an effort to change the paradigm of PRO assessment to measure the information most relevant to patients in clinical trials. This includes measuring treatment toxicity, symptom burden and physical function. This is relevant information that other patients want to know about a specific treatment for a specific disease to help them in their treatment decision making. The voice of the patient is critical to assess these items, through PRO measurements. In order to ensure that PRO questionnaires are filled out during a clinical trial, there are ways to make them more acceptable to patients by involving patients in their development (what questions are asked/how many questions are asked/when they are given to patients/how they are given to patients).

Patients can inform study teams on the acceptability, understandability and relevance of the questions being asked. Patients can be a key contributor to clinical trial development by identifying the questions that are most important to them and the best way to ask these questions during a clinical trial.

Patient advocacy and educational organizations like Cancer Information & Support Network (CISN) are advocating for changes in PRO collection in clinical trials. Based on information collected from many survivors, caregivers and advocates, a patient-centric clinical trial development model will be proposed. The model includes the involvement of patients as key stakeholders early in clinical trial development to incorporate PROs into clinical trials that are acceptable for patients to complete and the outcomes are meaningful and matter to future patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-18-04

Title: The advocates in science (AIS), a research advocacy program of Susan G. Komen®

Spears PA A, Finestone S, Jernigan C, Johnson P, Johnson J, Durham K, Wright K and Sabelko KA A. Susan G. Komen, Dallas, TX.

Body: Susan G. Komen® has a longstanding commitment to ensure that the unique and valuable perspectives of breast cancer patients, survivors, and co-survivors are integrated into the scientific dialogue and decisions impacting Komen's progress toward ending breast cancer. Komen established the Advocates in Science (AIS) Program to build a cadre of skilled, knowledgeable and broadly-networked breast cancer research advocates and engage patient advocates throughout the research process, where they provide a real-world understanding of what matters most to patients and a sense of urgency to find more-effective ways to prevent, diagnose, treat, and ultimately cure breast cancer.

The AIS Program, overseen by an AIS Steering Committee, takes a multi-pronged approach to research advocacy that includes the following components:

Involvement in Komen's Research Grant Strategy and Peer Review: Through the AIS Program, Komen matches advocates to Komen activities that best fit their training, experience and preference. A few of our most experienced breast cancer advocates serve on Komen advisory boards, providing strategic guidance and input on the research grant focus, and many others participate as advocate reviewers of our research grants.

Engagement of Advocates in Komen-funded Research Projects: Advocate involvement is required in most Komen-funded grants and strongly encouraged in all other Komen-funded research projects. Through the Program, Komen has created several tools – some aimed toward advocates and others targeted toward researchers – to facilitate effective advocate engagement in research projects.

Education & Training: Komen is committed to developing the next generation of breast cancer research advocates and strengthening the skills and knowledge of all AIS members. The AIS Program provides opportunities for advocates to learn more about breast cancer research and strengthen their research advocacy skills through a variety of educational sessions and rigorous training programs. These sessions/programs vary in format, from online tools, webinars, face-to-face meetings, and peer-to-peer mentoring.

Communication: The AIS Update keeps advocates up-to-date on recent scientific advances in breast cancer; Komen's research and its impact; and upcoming educational and participation opportunities for advocates both within Komen and nationally. Engaging patient advocates is increasingly being recognized as a fundamental factor in developing, implementing, and overseeing research that more effectively meets patients' needs. With its rich and growing experience in research advocacy, Komen is one of the pioneers of "patient-centric" research programs. Through the AIS Program, Komen continues to build a cadre of knowledgeable and experienced research advocates who bring the patient voice to Komen's – and other organizations'—research programs, in all areas of breast cancer research from the bench to the bedside to the curbside and back.

AIS members are also active in their communities and serving as bridges between advocates, scientists and Komen Affiliates to enhance understanding and excitement about research; foster greater support for research and its funding; build stronger researcher<->community connections; and energize hope for finding cures.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-18-05

Title: Male breast cancer— infusing a little blue into the sea of pink

Wagner H and Boyages J. A Man's Pink (Male Breast Cancer Advocacy Organization), Brooksville, FL and Macquarie University, Sydney, NSW, Australia.

Body: Male breast cancer (MBC) is a rare disease dominated by a sea of pink. Issues faced by men with this disease include delayed diagnosis, lack of male-specific information, stigma about having a "female disease" and often under-treatment. MBC is often treated as a female breast cancer but differences between the two are starting to emerge. Men are often older at diagnosis and sometimes considered "too old" for more aggressive treatments such as chemotherapy. Drugs such as aromatase inhibitors may not be as effective in men as in women but are often prescribed. Drugs such as tamoxifen can cause side-effects such as weight gain, hot flushes, loss of libido and impotence.

Support for patients with MBC is less advanced than that for female breast cancer. A Man's Pink, a MBC advocacy organization, mission is to promote MBC awareness, increase early detection, optimize and increase the survival rates for men diagnosed with breast cancer. *Male Breast Cancer: Taking Control* (BC Publishing, Boyages, 2015) empowers patients to understand their diagnosis and treatment.

Apart from incidence data, prognosis and treatment options, this talk will focus on how a website (www.malebreastcancer.ca) dedicated to MBC can help dismiss some of the myths and help overcome stigmata for men facing difficulties at diagnosis, treatment and recovery.

Our goal is to promote awareness to assist with earlier diagnosis and treatment to improve survival rates and the journey for men in their battle with breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-19-01

Title: Non-metastatic and metastatic breast cancer patients' priorities when considering a treatment decision

Buzaglo JS S, Miller MF F, Longacre M and Kamal AH H. Cancer Support Community Research & Training Institute, Philadelphia, PA and Duke Cancer Institute, Durham, NC.

Body: Introduction: Health care value is of increasing importance in light of rising health care costs. Several frameworks have been developed to measure value in cancer care. Yet, the patient perspective of value is not fully understood or integrated into such measurement. Understanding patient priorities when deciding upon treatment would contribute to effectively measuring and communicating value. To contribute further to patient perspective on value, we examined patient priorities when deciding upon treatment between non-metastatic breast (BC) and metastatic breast (MBC) cancer patients. We focused on these populations to explore if differences exist among populations likely to experience differences in care and related costs due to differing trajectories. **Methods:** From April-May 2016, 221 BC and 91 MBC survivors in the online Cancer Experience Registry answered questions about treatment decision-making. Using a 5-point Likert scale (not at all to very much), respondents rated the importance of (1) financial cost of care, (2) length of life (LoL), (3) quality of life (QoL), and (4) impact on family, when making a treatment decision. They also ranked the same factors in order of importance (1 most to 4 least important). Finally, they considered two factors at a time indicating which had greater impact on their decision. Chi², independent t-tests and Wilcoxon rank-sum tests were used to investigate differences between BC and MBC. If there were no significant differences, combined results are presented. **Results:** Participants were median age 56 y; 88% non-Hispanic white. Characteristics differed significantly between BC and MBC: 41% and 31%, respectively, working full-time; 8% and 31% on disability; 35% and 50% income < \$60K; and median time since diagnosis 3 y and 4.5 y. The greatest proportion of registrants (80%) indicated QoL was "very much" important when deciding upon treatment followed by LoL (59%), family impact (56%), and cost (23%). The mean (±SD) rank score of factors corroborated the order of importance: QoL (1.6±0.9); LoL (2.1±1.0); family impact (2.3±1.0); and cost (3.1±1.2). When making pairwise comparisons between QoL, LoL, and cost, 40% of BC survivors indicated LoL was much more important than cost vs. 52% among MBC (chi²=4.1, p=0.042), and 61% of BC indicated QoL was much more important than cost vs. 72% among MBC (chi²=3.6, p=0.058). **Conclusion/Implications:** When considering treatment, BC and MBC patients prioritized QoL suggesting the need for conversations with clinicians to focus on QoL among other factors. Both BC and MBC patients gave less priority to cost than other factors. Interestingly, MBC patients placed an even greater emphasis than BC patients on QoL and LoL relative to cost. Given the rising cost of care, healthcare teams may also need to prompt discussions around potential financial toxicity particularly among a population likely to be faced with long-term costs such as MBC. Future research should explore how to develop, evaluate and implement evidence-based tools that enhance doctor-patient communication around QoL and cost.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-19-02

Title: Young women with early stage breast cancer and their supportive care needs: Results from a regional survey

Ratcliffe J, Hodgson N, Rana P, Forbes M, Levine M and Sussman J. McMaster University, Hamilton, ON, Canada and Hamilton Health Sciences, Juravinski Cancer Centre, Hamilton, ON, Canada.

Body: Background

Approximately 18% of all newly diagnosed breast cancer cases in Canada occur in women less than 50 years of age. Young women with breast cancer (YWBC) may experience unique physical and psycho-social issues yet there is a lack of data outlining their specific needs and concerns across the trajectory of care. This study described the unmet supportive care needs of YWBC at a regional cancer centre in southern Ontario, Canada.

Objectives

1. To describe unmet supportive care needs of young women (<45 years) with early stage breast cancer in a representative region by using the Supportive Cancer Needs Survey (SCNS-SF34).
2. To describe the level of satisfaction with information to support cancer treatment decision making and causes of distress among YWBC (<45 years) in a representative region.

Study Design

This study used a prospective survey design that was administered to consenting YWBC. The Supportive Care Needs Survey (SCNS-SF34) was used to measure respondents' need for cancer support and care. This instrument captures needs through 34-items that cover five domains: psychological needs, health system and informational needs, physical and daily living needs, patient care support needs and sexuality needs. In addition, an original 26-item survey questionnaire was administered. Survey items were developed from the recurring themes of an earlier project that informed 4 levels of inquiry: demographics, decision making/ informational support, disease and treatment characteristics, and causes of distress.

Results

Fifty-one patients were approached by a member of their circle of care. Of these patients, 35 completed the survey resulting in a 69% response rate. The majority of respondents were between the ages of 33 and 40 (48.6%), were diagnosed within 6 to 12 months of study entry (60.0%); had a university degree (51.4%); were married (82.9%) and (65.7%) have young children. The three most common Moderate to High Unmet Supportive Care needs (rated 4 or 5 on a 5-point Likert scale) were: "Worries of those close to you" (34.3%), "Fears about the cancer spreading" (34.3%), "Anxiety" (28.6%) all of which reside in the psychological domain. Overall respondents agreed or strongly agreed to statements regarding their level of satisfaction with information to support cancer treatment decision making provided by their oncology team. Respondents did score lower in satisfaction with the information their oncology team provided regarding fertility. In terms of psychosocial support, 40% percent of respondents reported they had met with a social worker and 17% reported attending a breast cancer support group.

Conclusion

The results highlight that the supportive care needs of many YWBC are unmet, particularly those related to psychological burden and fertility concerns. Early identification and appropriate referral to fertility specialists and/or supportive care social workers could improve current practice. Further research is needed to explore how barriers to fertility and supportive care needs of young adults with cancer may best be overcome.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-19-03

Title: A meta-analysis of cognitive impairment in breast cancer survivors treated with chemotherapy

Bernstein LJ J, McCreath GA A and Rich JB B. Princess Margaret Cancer Centre, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada and York University, Toronto, ON, Canada.

Body: Background: Cognitive impairment in women with breast cancer has long been attributed to chemotherapy, but the evidence for this has been inconsistent, and more recent research suggests that additional cancer- and treatment-related factors also impact cognition. To systematically evaluate and describe “chemobrain,” a multilevel meta-analysis of objective cognitive function in breast cancer survivors treated with chemotherapy was conducted to estimate the magnitude of impairment across 9 cognitive domains and explore moderating variables.

Method: PubMed, Embase, CINAHL, Cochrane CENTRAL, Scopus, and PsycINFO were searched from their inception to May 2016 to identify studies that compared the cognitive performance of survivors treated with chemotherapy to a control group or to their own prechemotherapy testing. Multilevel modelling was used to account for the interdependence among effect sizes obtained within the same study.

Results: Seventy-seven studies involving 3417 cancer patients treated with chemotherapy yielded 1548 effect sizes. Cross-sectional comparisons revealed worse overall cognitive performance in survivors treated with chemotherapy relative to healthy controls ($d = -0.27$, 95% CI [-0.32, -0.21]) but not disease-specific controls ($d = -0.07$, 95% CI [-0.14, 0.00]). Relative to all controls, cognitive impairment in survivors treated with chemotherapy was observed in tests assessing language ($d = -0.25$), executive function ($d = -0.21$), processing speed ($d = -0.15$), delayed recall ($d = -0.15$), and attention/concentration ($d = -0.14$), but not in visual/spatial perception, immediate recall, recognition memory, or psychomotor domains. Because patients and controls were not well matched in many studies on age, education, and IQ, which are known to impact cognitive test performance, group differences in these variables were explored as moderators of observed cognitive deficits in post hoc analyses. Results showed that chemotherapy patients and controls performed equivalently when the controls were 6 years older or had 1 less year of education. Longitudinal comparisons showed improved postchemotherapy performance relative to prechemotherapy baseline, but practice effects were not accounted for in these comparisons.

Conclusion: Breast cancer survivors treated with chemotherapy show subtle yet diffuse cognitive impairment. Their cognitive performance was equivalent to that of women in comparison groups who were 6 years older, which suggests that chemotherapy accelerates cognitive aging. Results from breast cancer patients who were not treated with chemotherapy, however, indicate that chemotherapy is not the only determinant of cognitive dysfunction. Group differences in age and education moderated the magnitude of cognitive impairment. The cognitive reserve framework, which posits that individuals differ in the degree of neural disruption they can withstand before exhibiting cognitive deficits, should be considered in future studies in order to better understand moderating effects on cancer-related cognitive dysfunction. In particular, both cross-sectional and longitudinal studies should ensure that comparison cohorts are closely matched on age, education, and other relevant cognitive reserve variables.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-19-04

Title: Psychosocial distress monitoring in a multidisciplinary, inner city breast center

Castaldi M, Elrafei T and Soliman C. Jacobi Medical Center, Bronx, NY.

Body: Objective: Beginning in 2015, all Commission on Cancer–accredited cancer programs must have developed and implemented a process to screen for psychosocial distress and provide appropriate psychosocial care. At our institution that serves uninsured and underrepresented minority populations, we integrated a distress tool on a continuum, rather than as a onetime event. We reviewed the initial screening tools from our breast center to determine how psychosocial distress differs throughout treatment in order to identify pivotal times during course of treatment and to prioritize intervention strategy.

Methods: NCCN distress thermometer was administered to each patient with review of stressors to complete at 4 different intervals after diagnosis with breast cancer. Newly diagnosed breast cancer patients were asked to complete distress screening at various phases of their cancer care: shortly after diagnosis at surgery office visit, after surgery at first chemotherapy cycle, third medical oncology office visit, and last chemotherapy cycle. The tools were administered by patient navigators and referred to social work with a distress score of 5/10 or higher. Tools were then scanned into the medical record and collected by the social worker. We reviewed the collected distress tools and compared the data based on the where the patient was in course of treatment. Identifying stressors were grouped into 4 categories: practical, family, emotional, and physical.

Results: in April 2015, the implementation and preparation of distress screening was begun. Between August 2015 and April 2016, 94 distress tools were completed by patients in our breast center. 42.5 % patients had a distress score of 5 or above at their initial screen at surgical consultation, 31.8 % at their first cycle of chemotherapy, 53.3% at third medical oncology office visit and 50 % at completion of chemotherapy, triggering a social work intervention. The most common stressors were emotional, worry and fear, (44%) at initial assessment and physical, fatigue and tingling in hands and feet, (100%) at last chemotherapy cycle, with family stressors 33.3 %, and practical matters 28%. The least common stressor was treatment decision or need for treatment change. Mean stress levels were 8/10 for surgical patients and 6/10 for patients seeing medical oncology. Although 4 screens were performed, the spectrum of stressors did not change significantly over continuum of care other than all patients reporting fatigue with a score of 5 or higher at completion of chemotherapy. All patients had psychosocial barriers to care that caused stress regardless of phase of cancer care.

Conclusion: Patients are at their peak stress levels at the time of diagnosis in consultation with the surgeon. As they move along their treatment plan, stress levels decrease, but the stressors seem to remain the same, except for physical stress being highest at end of chemotherapy. We recommend timing of the administration of the distress tools be based on capturing peak stress (at time of diagnosis) and then again at completion of chemotherapy to monitor for stress reduction. Addressing psychosocial barriers seems to be paramount in lowering distress scores in our underrepresented, minority breast cancer population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-19-05

Title: Efficacy of screening and treatment of breast cancer patients reporting high level of distress

Daneker SR R, Bendinger GM M, Thomas JW W, Smith E, Kendrick D, Hartman S, Gordon C, Barber K, Langlois C, Pabbathi H, McKnight JE E, Johnson AT T, Lammersfeld C, Denny D, Markman M and Alvarez RH H. Cancer Treatment Centers of America, Newnan, GA and The University of Georgia, Athens, GA.

Body: Background: Cancer patients (pts) are burdened by symptoms related to the disease itself or to the toxicities of treatment. A recent meta-analysis has shown that anxiety is the most common mental health issue among cancer survivors [Mitchell AJ, 2013]. The ASCO clinical oncology guideline adaptation recommends all health care providers routinely screen for the presence of emotional distress and symptoms of anxiety from the point of diagnosis onward [Andersen BL, 2014]. Consensus-based recommendations have been published to help cancer centers meet the American College of Surgeons Commission on Cancer's accreditation requirement to screen for distress [Pirl, 2014] At our comprehensive community cancer center we perform distress screening using the M.D. Anderson Symptom Inventory (MDASI), composed of 27 questions. Pts who report moderate and severe levels of distress (≥ 5) on the MDASI are identified and referred for therapeutic interventions offered by the facility's integrative oncology services.

Materials and Methods: The MDASI is an assessment tool that captures pts' perceived symptom burden for real-time clinical intervention, taken at the point of no intervention (baseline) and every 21 days or greater. The 27-question MDASI is comprised of the M.D. Anderson Symptom Inventory (MDASI), a validated 19-item assessment instrument, with a Symptom Inventory Tool (SIT) added by our center of 8 questions and a free-text box. Symptoms are rated "at the worst" on an 11-point numeric scale ranging from 0 ("none present") to 10 ("as bad as you can imagine"), as experienced by the patient in the past 24 hours.

Results: Over a ten-month period (9/1/2014 to 6/30/2015), 247 breast cancer (BC) pts completed the MDASI at intake and again ≥ 21 days after. Analysis of their initial surveys identified 69 pts (27.9%) who rated their distress as ≥ 5 (1st MDASI mean = 6.83), scores which would have initiated a support system response with referrals to integrative medicine services for intervention. Second MDASI results from these 69 BC pts revealed an average 2.29 distress score reduction (2nd MDASI mean = 4.48), with 51 pts (73.9%) reporting a decrease in distress, 7 pts (10.1%) having no change, and 11 pts (15.9%) reporting increased distress. More specifically, the group with decreased distress levels documented a mean distress score of 3 on their 2nd MDASI, averaging a significant 4-point diminution of distress for three-fourths of the BC pts heavily burdened by this symptom. The integrative services most utilized by these pts were Nutrition Therapy (100%), Spiritual Care (96.1%), Mind-Body Counseling (82.4%), Rehabilitation Therapy (41.2%), Acupuncture (35.3%), and Massage Therapy (35.3%).

Conclusions: Distress is a relevant symptom reported by cancer pts. This study demonstrates that early intervention in BC pts using integrative oncology approaches reduced distress in 74% of cases.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-19-06

Title: Return to work after breast cancer diagnosis: An observational prospective study of 125 patients in South America

Landeiro LG G, Fraile NMP MP, Fede AB B, Fonseca LG G, Testa L, Petry V, Gonçalves MS S, Costa RDP DP, Cohn DB B, Ferrari MM M, Sunahara RS S, de Gouveia ACC C and Mano MS S. Instituto do Câncer do estado de São Paulo, São Paulo, Brazil.

Body: Background: Breast cancer is the most common cancer in women. While its incidence has been increasing, recurrence and mortality rates have been decreasing. Because of that, cancer can now be regarded as a transient shock that does not prevent the survivors to live normal lives, including returning to their workplace. From a social perspective, long periods of sick leave have a strong economic impact. From the patient perspective, prolonged sick leave can cause financial difficulties and emotional distress. In United States and Europe, RTW rates vary among breast cancer patients from 24-66% after 6 months and 53-82% after 36 months of diagnosis. Factors associated with the decision to return to work are: age, chemotherapy, sequelae related to cancer therapy and support from the employer and coworkers. However, these findings vary among the different populations studied, suggesting that other factors may also interfere with the decision to return to work. Data on RTW after breast cancer diagnosis is not available in South America nor in developing countries – where the workforce tends to be relatively younger than average. The primary objective of this study was to evaluate RTW rates on months 12 and 24 after breast cancer diagnosis, and to evaluate factors associated with the decision to return to work.

Methods: Prospective, observational study evaluating RTW rates in patients diagnosed with breast cancer, > 18 and <57 years old (in Brazil women can retire at age 60) and under remunerated work for at least 03 months at the time of diagnosis. Patients with metastatic disease were excluded. Based on previously data, we estimated that 125 patients would be required to achieve the primary endpoint. Each patient was followed for 02 years. On months 6, 12 and 24 they answered a telephone interview and the FACT-B questionnaire. Disease characteristics and treatment were collected from electronic medical records. After completion of all third row of interviews (by september, 2016) we will perform univariate and multivariate analysis by logistic regression method to determine the independent predictors of return to work.

Results: Between july, 2012 and september, 2014, 125 patients were enrolled. The median age was 45.1 (range 25-57). Half of the patients (52.9%) were married at the time of diagnosis, and 96.9% received support from their life partner. Most of them reported that they liked their job (93.8%) and received support from their employer (61.5%), but only 29.1% reported adjustment offering, so that they could keep working during treatment. Almost half of patients (47.6%) had stage II disease, 75.4% and 19.7% were HR and Her 2 positive, respectively, and 92.6% received chemotherapy as part of their treatment. Overall, 22.1% and 28.8% of patients returned to work, at 6 and 12 months after breast cancer diagnosis, respectively.

Conclusion: Twelve months after breast cancer diagnosis, less than 30% of the patients had returned to work. By september, 2016, we will have completed the “24 month” interviews and will present RTW rates at that time and also correlative analyses of factors that affected the RTW in this population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-19-07

Title: Fertility concerns in young women after diagnosis of breast cancer: Experience of a breast cancer unit in México

Campos-Gomez S, Campos-Gomez KA, Ovando-Trujillo A and Palacios-Gutierrez F. Centro Oncologico Estatal ISSEMYM, Toluca, Mexico.

Body: Background

Nearly 30% of all new breast cancer diagnoses occur in pre-menopausal women and about 7% of diagnoses occurring in women aged 40 years or less. Breast cancer treatment can increase the risk of early menopause and infertility. It is the major source of distress and concern form many young breast cancer patients who have no finished their families and have plans for childbearing.

Methods

As part of multidisciplinary approach in the Breast Cancer Unit at Centro Oncologico Estatal ISSEMYM an evaluation psychosocial aspects of sexuality, fertility and menopause after diagnosis of breast cancer has been implemented.

We conducted an exploratory study to better understand fertility concerns, the role of fertility in the treatment decisions and chemotherapy acceptance in a cohort of young women newly diagnose breast cancer evaluated in a Breast Cancer Unit. We invited eligible women to participate, fertility concerns was the primary end point

Results

A total of 52 women were evaluated. Of these, we exclud 2 patients they have stage IV disease at re staging. Thus 50 women were included in the analyses. Median age at diagnosis was 36 years (range 23 to 40 years). 86% had children already at the time of breast cancer diagnosis. Only 4 participants (8%) reported that before their breast cancer diagnosis, they wanted to have future or additional children. Whereas forty six (92 %) did not. Of these patients 85% don't want to have children because of prior decision and 20% because fear of cancer recurrence. Forty nine patients would agree to receive chemotherapy (98%) and 51% would accept a risk of infertility of 76-100%. This acceptance was dependent on already having children. 68% would accept it only for $\geq 20\%$ gain in cure.

Conclusion

Our study represents one of the first prospective evaluations of fertility concern in young women with recently diagnosed breast cancer in a Public Hospital in México. For the majority of young patients with breast cancer, cure remains their first priority. Most of the women have child before diagnosis and only 8% want to have additional children.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-20-01

Title: Implications of financial modeling in breast cancer clinical research from 1990 to 2010

Jerez Y, Lopez-Tarruella S, Marquez-Rodas I, Perez S, Ocaña A, Echavarria I, Lobo M, Gallego I, Torres G, Ortega L, Garcia G, Palomero I, Gonzalez Del Val R, Massarrah T, Esteban M, Del Monte-Millan M and Martin M. Hospital Gregorio Marañón, Madrid, Spain and Yale University, Albacete University Hospital, New Haven, Albacete.

Body: SUMMARY: Over the past two decades significant progress has been made in breast cancer treatment resulting in a substantial improvement in patients' outcome. But we have to think about who promotes all this research and the consequences of the type of funding. This project aims to evaluate the implication of finance in clinical research and the variance according to the type of funding.

OBJETIVES: To evaluate the financial evolution of breast cancer clinical trials in the past two decades, regarding the phase of development design of the studies, the collaboration between Academy (Acad) and Industry (Ind), the sample size, the study results and the statistical analyses conducted.

METHODS: A systematic review was performed using MEDLINE to identify breast cancer randomized clinical trials published between January 1990 and December 2010. Studies that involved chemotherapy, endocrine and/or targeted therapies, where the primary endpoint was considered adequate to support a drug approval in oncology according to the FDA and EMA (U.S. Food and Drug Administration and European Medicines Agency, respectively), were included.

RESULTS: Data were evaluated 2,211 and 472 met selection criteria comprised in the methodology. During the first decade the Acad was the main breast cancer research promoter being replaced by the Ind. throughout the second decade ($p < 0.0001$). Thirty nine percent of the studies evaluated were phase III (39% Acad, 61% Ind), 15% were phase II (30% Acad, 70% Ind) and the remaining 47% were not classified by authors (65% Acad 35% Ind). As for the primary endpoint, 25% of the phase III trials evaluated progression free survival, 15% overall response rate, 1% time to progression and only 5% examined overall survival. Sixty five percent of the trials were national (60% Acad 40% Ind) and 35% international (25% Acad 75% Ind). Single-center studies accounted for 11% of the trial (65% Acad 35% Ind). Most of the national trials were developed by the US. Fifty four percent of the studies were conducted by research groups (67% supported by Ind. and 33% Acad.). The Ind sponsored 26% of the studies in the first decade and 50% during the second. The median number of patients enrolled by research groups was 892 in contrast with 409 included by other organizations. The primary endpoint was achieved in 19% of the Acad trials and 21% of the Ind trials. Only 53% of the studies declared intention to treat based analysis in their statistical workout.

RESULTS

		ACADEMY(%)	INDUSTRY (%)	P
PROMOTION OF THE STUDY	1990-2000	121(26)	68(14)	0,0001
	2001-2010	105(22)	178(38)	0,0001
STUDY DESIGN	UNICENTRIC TRIALS	34(7)	18(4)	0,007
	MULTICENTRIC TRIALS	191(40)	228(48)	
	NATIONAL TRIALS	183(39)	122(26)	0,0001
	INTERNATIONAL TRIALS	42(9)	124(26)	
	COOPERATIVE GROUP	95(20)	160(34)	
	NOT COOPERATIVE GROUP	130(28)	86(18)	
STATISTICAL ANALYSIS	INTENT OF TREAT	86(18)	163(35)	
	NOT DECLARATED	140(30)	83(18)	

CONCLUSIONS: There is a significant tendency towards the promotion of research by the pharmaceutical industries during the last two decades, leading a change in the clinical trials design and the endpoints.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-20-02

Title: Value of cancer care for metastatic breast cancer patients and providers

May SG G, Chung AH H, Vania DK K, Hou N, MacEwan J, Batt K, Kurian AW W, Zacker C, Globe D and Goldman DP P. Precision Health Economics, Los Angeles, CA; Wake Forest Baptist Health, Winston-Salem, NC; Stanford University, Stanford, CA; Novartis Oncology, East Hanover, NJ and University of Southern California, Los Angeles, CA.

Body: Background: The landscape of treatment options and associated prognosis for patients with metastatic breast cancer (MBC) is rapidly evolving. In response to these advances in therapy, numerous organizations have invested considerable resources into developing evaluation frameworks seeking to clarify the value of new therapies. While some of these frameworks foster patient-provider shared decision making, others are more payer focused, and all are limited in their incorporation of patient perceptions of value and evidence on treatment aspects most meaningful to patients.

Objectives: 1) To identify the attributes of treatment that patients with MBC value most, and 2) to explore the alignment between patient valuation of treatment attributes and healthcare provider perceptions of what patients value.

METHODS: Four 90-minute focus groups were conducted: two with patients (aged <50 years and aged ≥50 years) and two with healthcare providers (oncologists and oncology nurses) who treat patients with MBC. Using semi-structured discussion guides tailored to each participant group, patient and provider perceptions of the factors most important to patients when considering treatment were explored as well as various sources of perceived value in cancer care. Discussions were audio recorded and transcribed. Thematic analysis identified attributes patients with MBC consider when making treatment decisions, and concordance between patients and healthcare providers was assessed.

Results: A total of 24 patients and providers (n=5 patients <50 years, n=5 patients ≥50 years, n=7 oncologists, and n=7 nurses) participated in four different focus groups. The factors of greatest importance to patients included: impact of treatment side effects on daily life, depth of treatment response, longevity of life, and the value of hope in traversing their illness and achieving survival landmarks and goals. In contrast, oncologists focused predominantly on clinical considerations, such as treatment effectiveness and managing side effects. Oncology nurses noted similar clinical factors as oncologists, but also aligned more closely with patients on humanistic elements informing treatment decision-making.

Conclusion: This analysis reveals that while patient and healthcare provider assessments of value in treating MBC are well-aligned with respect to clinical factors such as managing side effects and depth of treatment response; patients also prioritize emotional and psychological factors, -- like having hope and avoiding suffering -- in addition to clinical factors. Moving forward, patient-centered value frameworks for MBC will need to address this gap between what providers and payers value and patient goals and priorities.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-20-03

Title: Collateral damage from metastatic breast cancer – Preliminary results

Love SM M, Bernstein L, Obidegwu A, Ottenbacher A, Eshraghi L and Clague J. Dr. Susan Love Research Foundation, Encino, CA; Beckman Research Institute, City of Hope Comprehensive Cancer Center, Duarte, CA; 2M Research Services, Fort Worth, TX and Beckman Research Institute, City of Hope, Duarte, CA.

Body: BACKGROUND: Historically, research efforts have focused on developing new treatments, education, and support programs for metastatic breast cancer (MBC) patients. Less emphasis has been placed on understanding quality of life (QoL) issues for this underserved population. However, when faced with a metastatic diagnosis, managing, and minimizing the collateral damage resulting from sequential and often continuous treatments is critical to improving quality of life. Through this preliminary analysis, our aim is to understand the unique experience of MBC and identify potential areas of critical need.

METHODS: The Health of Women (HOW) Study™ is an online study for women and men, with and without a history of breast cancer, aimed at identifying causes of breast cancer. The (HOW) Study periodically releases new questionnaires and is currently comprised of ten questionnaires collecting information on personal and family health history, lifestyle, environmental exposures, breast cancer diagnosis and treatment, and QoL. Over 53,000 people are enrolled in (HOW), of which, 11,508 have completed the QoL questionnaire. Areas covered included chronic conditions, symptoms, moods and feelings, reproductive health, daily activities, patient-provider communication, and social and financial concerns. QoL measures were compared between participants with no history of breast cancer (NoHx), with non-metastatic breast cancer (non-MBC), and with MBC.

RESULTS: Of the 11,508 respondents, 3,965 reported a diagnosis of breast cancer—of those, 205 were metastatic. The majority were female (99%) and non-Hispanic White (95%). MBC were younger (28% < 50 years, 65% 50-69 years) than non-MBC (20% < 50 years, 69% 50-69 years; $p < 0.05$). Overall, participants with MBC reported a lower QoL (20% excellent, 10% poor) than non-MBC (41% excellent, 3% poor) and NoHx participants (44% excellent, 2% poor; $p < 0.05$). MBC and non-MBC participants did not differ on hot flashes, vaginal problems or fertility concerns. However, MBC participants were more likely than non-MBC participants to report: 1) Symptoms — including digestive, mouth and nose, hair and skin, eye, pain, fatigue and night sweats — significantly affecting their lives; 2) Problems with concentration, mood swings, anxiety, depression, memory, and sleeping; 3) Attributing each symptom and/or problem to their breast cancer treatment; 4) Problems performing daily activities; 5) Better communication with their clinical team; 6) Severe financial concerns including difficulty with medical expenses, losing one's job and/or health insurance, not meeting the financial needs of one's family and not being able to pursue career of choice; and 7) Severe social concerns including feeling dependent, isolated, and being treated differently (all p -values < 0.05).

CONCLUSIONS: Participants with MBC had a considerably diminished QoL compared to non-MBC and NoHx participants. We identified key areas of concern and multiple areas of collateral damage not standard in research assessment. Our next steps are to use these data to develop a formal, qualitative questionnaire that includes newly identified collateral damage issues, and expand our sample of MBC. The combined data will guide us in developing recommendations for improving the QoL for people with MBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-20-04

Title: Young women with breast cancer in Mexico: A report of the pilot phase of the “Mujer Joven y Fuerte” prospective cohort

Villareal-Garza CM M, Platas A, Castro-Sánchez A, Miaja M, Bargalló-Rocha E, Martínez-Cannon BA A, Vega Y, Fonseca A, Ramos-Eliás P, Márquez-Perez CJ J, Bukowski A, Goss P, St. Louis J, Chapman J-A, Partridge A, Meneses A and Mohar A. Instituto Nacional de Cancerología, Mexico City, CDMX, Mexico; Program for Young Women with Breast Cancer, Mexico City, CDMX, Mexico; Breast Cancer Center, Tecnológico de Monterrey, Nuevo León, Monterrey, Mexico; MILC , Medicos e Investigadores en la Lucha Contra el Cáncer de Mama, Nuevo León, Monterrey, Mexico; MGH-Avon Breast Cancer Program, Massachusetts General Hospital Cancer Center, Boston, MA; (Retired) Canadian Cancer Trials Group, Queen's University, Kingston, ON, Canada and Dana-Farber Cancer Institute, Boston, MA.

Body: Background: Despite high rates of breast cancer in young women from low-and-middle-income countries (LMICs), their needs and concerns are not systematically studied or addressed. Understanding the characteristics of young women with breast cancer (YWBC) and the issues they face is of great relevance to the medical community, in order to tailor clinical interventions and supportive care for this unique and understudied patient population. The Mexican cohort “*Mujer Joven y Fuerte*” (Young and Strong Woman) has the goal of comprehensively characterizing and assessing the needs of YWBC in Mexico using patient- and physician-based surveys. **Methods:** A prospective cohort of newly diagnosed YWBC was established in November 2014 at two Mexican cancer centers in Mexico City and Monterrey. Eligible women answer web-based surveys on relevant topics including physical activity, genetics, psychosocial needs, and fertility. Clinicians complete pre-specified surveys using the US NIH BOLD Task Force common data elements registering clinical/pathologic characteristics and outcomes. Patients are evaluated at diagnosis, after 6 months, and annually for 5 years. Sub-studies assessing changes in cognition, sexual function and satisfaction, quality of life and depression/anxiety are being conducted, and biologic samples are stored for future research. **Results:** 96 YWBC with median age at diagnosis of 34 (21-41 y) were accrued to our pilot phase. 26% were single and 25% childless. 43% had higher education and 28% were employed. 90% presented with a self-detected mass. Clinical stage at diagnosis was distributed as follows: stage 0: 2%; I: 15%; IIA: 13%; IIB: 17%; III: 47%, and stage IV: 6%. The most frequent molecular subtype was HR+/HER2- (47%), followed by HER2+ (26%) and triple negative (21%). First follow-up results will be available shortly. **Conclusions:** To our knowledge, this represents the first prospective cohort of YWBC in Latin America. We are expanding this project to other centers in the region. Our findings will help develop culturally tailored interventions aimed at improving the psychosocial and medical outcomes of this vulnerable patient population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-20-05

Title: Patient-reported cosmetic outcomes in older breast cancer survivors: A population-based survey study

Swanick CW W, Lei X, Xu Y, Shen Y, Goodwin NA A, Giordano SH H, Hunt KK K, Jagsi R, Shaitelman S, Peterson SK K and Smith BD D. The University of Texas MD Anderson Cancer Center, Houston, TX and The University of Michigan, Ann Arbor, MI.

Body: Background: For older women with breast cancer, local therapy options may include (1) lumpectomy followed by whole breast irradiation (Lump+WBI), (2) lumpectomy followed by brachytherapy (Lump+Brachy), (3) lumpectomy followed by endocrine therapy alone without radiation (Lump alone), (4) mastectomy without radiation (Mast alone), or (5) mastectomy followed by radiation (Mast+RT). For many patients, several of these options are acceptable based on current guidelines, but little is known about the impact of treatment choice on long-term cosmetic outcomes. We surveyed a population-based cohort of older breast cancer survivors treated with 1 of these 5 options to assess patient satisfaction with cosmetic outcome.

Methods: We used nationally comprehensive Medicare claims to identify women age ≥ 67 diagnosed with non-metastatic breast cancer in 2009, treated with 1 of these 5 treatment options, and still alive in 2015. From this cohort, 1650 patients (330 patients per local therapy) were randomly selected. Of these, 397 opted out, and the remaining 1253 potential participants were mailed a survey that included the CanSORT Satisfaction with Breast Cosmetic Outcomes instrument (5-point scale, higher score indicates greater satisfaction) and the Breast-Q Satisfaction with Breast instrument (0-100 Rasch transformed score, higher score indicates greater satisfaction). Multivariable linear regression models were used to assess the association of local therapy with each outcome, adjusting for age, race, comorbidity, chemotherapy, patient-reported BMI, bra cup size, smoking, income, and education. Spearman's correlation assessed the relationship between the 2 outcomes. All analyses incorporated sample and response weights.

Results: We received completed surveys from 498 women (30% response rate). The median age was 73 years (range, 67-87 years). The interval from diagnosis to survey was 6 years for all patients. Among patients with evaluable CanSORT responses ($n=439$), the weighted mean score by treatment group was 3.64 for Lump+WBI, 4.01 for Lump+Brachy, 3.83 for Lump alone, 3.28 for Mast alone, and 3.25 for Mast+RT. In multivariable analysis with Lump+WBI as the referent, the adjusted CanSORT mean score was 0.37 points higher for Lump+Brachy ($P=0.009$), 0.35 points lower for Mast alone ($P=0.035$), and 0.33 points lower for Mast+RT ($P=0.048$). Among patients with evaluable Breast-Q Satisfaction responses ($n=418$), the weighted mean score by treatment group was 60.9 for Lump+WBI, 68.8 for Lump+Brachy, 66.7 for Lump alone, 58.8 for Mast alone, and 52.0 for Mast+RT. In multivariable analysis with Lump+WBI as the referent, the adjusted Breast-Q Satisfaction score was 7.4 points higher for Lump+Brachy ($P=0.03$) and 7.8 points lower for Mast+RT ($P=0.04$). Higher comorbidity predicted worse cosmetic outcome in both models; no other variables were associated with both outcomes. The 2 outcomes were highly correlated (Spearman's coefficient=0.8, $P<0.0001$).

Conclusion: In this nationally representative cohort, satisfaction with cosmetic outcome (as measured by 2 distinct instruments) was higher for patients treated with Lump+Brachy and lower for those treated with Mast+RT compared to Lump+WBI. These results may be used to inform patient treatment decisions.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-20-06

Title: Results of an educational program delivered through e-learning, focused on nursing for primary breast cancer patients

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Body: Aim: An educational program delivered through e-learning was conducted, focusing on nursing for primary breast cancer patients. This paper investigates the results of the program, clarifying changes in levels of understanding before and after the program, and evaluations of the program by nurses who participated. Methods: The content of the education program, which consisted of 24 lessons, each lasting 15-20 minutes, including case studies, covered concerns raised in questions by patients and families about diagnosis, multimodal treatment and nursing for primary breast cancer. Instructors were Certified Nurses in Breast Cancer Nursing and Certified Nurse Specialists in Cancer Nursing, and PowerPoint presentations with video and audio material were created. A dedicated website was created, and 1417 participants took the on-demand program, which was provided from September 2015 to March 2016. Survey participants were the first 500 nurses registered for the course to respond. A questionnaire created by the researchers was sent by post to respondents. Responses were anonymous with identifying information code, and questionnaires were sent with a return envelope before the program (within 2 weeks of registration) and after the program (within 1 month of the end of the program), with return of the questionnaire signifying consent to participate. The questionnaire comprised 24 items on level of understanding of breast cancer nursing, aligned to program content, plus 11 items on evaluation of the program. Responses were selected from a 5-point scale, where 1 meant "strongly disagree" and 5 meant "strongly agree". Analysis: Responses were quantified, and mean scores for each item were calculated. In addition, respondents were divided into two groups, namely, those with less than 3 years of breast cancer nursing experience (Group A) and those with 3 or more years' experience (Group B), and were compared using the Mann-Whitney U test. SPSS Statistics V22.0 was used. Ethical considerations: The study went through Research Ethics approval at the researchers' institute of affiliation. Results: A total of 126 people replied to both the pre-program and post-program questionnaire, and of these, the responses of 106 nurses (Group A: 34 nurses, Group B: 72 nurses) who were not qualified as Certified Nurses or Certified Nurse Specialists were used for analysis. Median age was 41.0 years (Group A: 38.5 years, Group B: 42 years), and median number of years of breast cancer nursing was 4.6 years. In terms of program evaluation, "I could study when it was convenient for me" scored 4.63, while "It will be useful for breast cancer nursing from now on" scored 4.57. In terms of comparison of level of understanding before and after the program, understanding improved on all 24 items, and was significant on 19 items. Comparison of the 2 groups showed significant difference on 2 items, "support for changes in body image" and "communication with the patient", with changes in level of understanding in Group A being greater. Discussion: The study suggests that participants' understanding of breast cancer nursing increased and improvements in clinical practice can be expected through e-learning materials that facilitate effective learning.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-20-07

Title: Research biopsies in oncology-patient willingness, perceptions, understanding, and experience: An integrative review

Abraham E, Spring L, D'Alleva J, Malvarosa G, Tripp E, Post K, Bardia A and Habin K. Massachusetts General Hospital, Boston, MA.

Body: Research Biopsies in Oncology – Patient Willingness, Perceptions, Understanding, and Experience: An Integrative Review.

Background

Tumors are classified into subsets based on molecular alterations to select personalized therapies, and research biopsies are essential to advance personalized medicine. However, research biopsies can be associated with complications, results might not directly benefit the patient particularly on-treatment biopsies obtained for evaluation of pharmacodynamics effect, and mandatory requirement can impact patient enrollment in clinical trials. Understanding patient perceptions and experiences surrounding research biopsies may highlight areas for improvement and could expand participation in clinical trials.

Objectives

The purpose of this integrative review explores and summarizes the current literature on oncology patient perceptions, willingness and experience of research biopsies evaluates data to determine potential implications for practice improvement, patient experience, and clinical trial participation.

Methods

Articles from January 2010-February 2016 were retrieved via a systematic search restricted to English of MEDLINE/Ovid. Search terms included *breast cancer, cancer, research, biopsy, perceptions and attitudes*. Inclusion criteria were studies that included patients with cancer and had information on patient willingness, perceptions, understanding, attitudes, and/or experience around research biopsies. Abstracts were independently reviewed for inclusion by two authors. For each selected manuscript, the following information was abstracted: primary author, sample and setting, results, conclusion, and assessment of strength of evidence level and quality using John Hopkins Nursing Evidence Based Practice (JHNEBP) Rating Scales.

Results

The search resulted in 175 unique studies. 17 abstracts were selected for full manuscript review. From these, 8 were excluded for not meeting inclusion criteria. The 9 selected studies ranged in sample size from 10-362 and all articles were rated IIIA-B evidence level. The majority of studies (66.7%) focused on breast cancer patients. Studies exploring patient willingness to undergo research biopsies (N=8) revealed variability in patient willingness to undergo research biopsies and identified research biopsies identified as a potential barrier to clinical trial participation. Studies exploring patient understanding of the risks and benefits of research biopsies (N=3) revealed overall poor understanding among patients. One study explored anxiety surrounding the research biopsy procedure, suggesting significant anticipatory anxiety is a significant concern. Issues surrounding informed consent were raised in several studies.

Conclusions

Research biopsies can be associated with significant anxiety, potentially impact enrollment in clinical trials, and consequently there is a need for development of strategies to improve patient experience and education. While a separate consent for research biopsies may be onerous, tailored educational materials, focus groups, as well as development of alternate strategies such as liquid biopsies, are essential to ensuring advancement in cancer care and outcomes.

Key Words: cancer, research, biopsy, perceptions, attitudes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-20-08

Title: Evaluation of a multidisciplinary cancer team quality in a breast cancer unit (BCU) in México

Campos-Gomez S, Campos-Gomez KA, García-Garces M, Hernández-Alvarez J, Serrano-Ortiz R, Machado-Reyes M, Fernández-Perez E, Morelos-Gomez G, Acuña-Arellano A, Medina-Castro JM, Gutierrez Balderas P, Cabañas Chavez NA, Luna Mendoza D and Ramirez Martinez P. Centro Oncologico Estatal ISSEMYM, Toluca, Mexico.

Body: Background:

The optimal management of patients with breast cancer (BC) requires the expertise of specialists from different disciplines. This has led to the evolution of multidisciplinary teams (MDTs), allowing all key professionals to jointly discuss individual patients and to contribute independently to clinical decisions. Data regarding BC MDTs in Latin-American countries are limited. The purpose is to assess the MDT performance in a Breast Cancer Unit in México.

Methods:

We conducted a prospective longitudinal study to evaluate the participation of MDT for breast cancer patients in Breast Cancer Unit at Centro Oncologico Estatal ISSEMYM. An assessor use a validated observational tool to rate quality and team decision-making for every patient discussed. Data were collected from a prospective observation of 150 breast cancer cases review during 3 months study period, in the Breast Cancer Unit meeting by a MDT. BCMDT (Breast Cancer Multidisciplinary Team) was integrated by medical, surgical and radiation oncologists, radiologist, pathologist, geriatrician and pharmacist. The information presented (patient case, radiological, pathological, comorbidities) and team member contribution and decision of further investigation or treatment as appropriate as well as MDT coordinators leadership role were analyzed. These tasks usually occur in a weekly meeting.

Results:

There were 150 breast cancer patients studied. The majority of the time for each case was taken up with information presentation, and often discussion occurred concurrently with presentation of information. The most common barriers to reaching clinical decisions were inadequate radiologic information (2 %) inadequate pathologic information (7%), and additional complementary studies needed (5%). The MDT had the ability to reach clinical decision in the 86% of cases. All members were present in the 98 % of sessions. The mean of participant was 12 in each meeting and the average time taken per patient was 3 minutes.

Conclusion:

This is an effort to collect information regarding BC MDTs from a Latin-American country and provides objective information of frequency, composition, function, and working mechanism of a BC MDT. The intervention of a MDT in the Breast Cancer Unit is efficacious and can improve decision making.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-20-09

Title: Diagnosing cognitive impairment (“chemo brain”) in breast cancer survivors

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Body: Background:

Cognitive complaints (“chemo brain”) are reported frequently after breast cancer treatment but little is known about its incidence and causes. Before we can intervene we need to be able to diagnose it properly. Mini Mental state examination (MMSE) can detect advanced Alzheimer's but won't help in detecting mild cognitive disturbances. We used Montreal Cognitive Assessment for Telephone (MoCA-T) and full scale Montreal Cognitive Assessment for Telephone (MoCA) to diagnose mild cognitive impairments in patients having normal MMSE.

Methods:

20 breast cancer survivors aged 50 years or older completed MoCA-T and MoCA, a year after they completed their treatment for breast cancer, describing the impact of their treatment regimen on their short term memory and ability to think and concentrate. On Day 5, they underwent a standardized laboratory protocol that assessed both behavioral and electroencephalographic (EEG) indicators of memory consolidation.

Results:

Patient characteristics:

20 postmenopausal breast cancer survivors aged 50 or above participated in the study a year after they completed their therapy. We are reporting *preliminary results* of 10 patients. Patients had stage I-III disease (stage I – One patient, Stage II – five patients, and stage III- four patients). All of them received various chemo regimens e.g. Cytoxan, methotrexate, 5-FU (CMF), Taxol, Trastuzumab (TH), Adriamycin (AC), taxotere, carboplatin (TCH). 8/10 patients received adjuvant radiation. 1/10 patient underwent reconstruction later. 7/10 patients received hormonal therapy with aromatase inhibitors.

Results of MoCA –T and MoCA:

MoCA-T, tests attention and concentration, executive functions, memory, language, conceptual thinking, calculations, and orientation. Possible scores range from 0-22 and scores <18 indicate mild cognitive impairment. The MoCA has possible scores from 0-30 and a score >26 is normal but <19 is indicative of cognitive impairment.

5/10 patients had abnormal MoCA and 3/10 patients had impaired MoCA-T signifying that almost 50%patients develop mild cognitive impairment after breast cancer treatment. During memory consolidation, EEG contained less theta and frequent bursts of alpha waves which is commonly seen in patients with neuropathic pain and insomnia.

MoCA-T and MoCA results for the patients

Patient no.	MoCA-T	MoCA
Ptient 008	17*	23*
Patient 010	21	26
Patient 011	19	23*
Patient 012	16*	27
Patient 013	20	27
Patient 015	17*	21*
Patient 016	18	24*
Patient 017	20	25*
Patient 019	19	26
Patient 020	21	29

*presents affected patient

Conclusions:

The study signifies that mild cognitive impairment from breast cancer treatment (or “chemo brain”) was frequently reported and MoCA-T and MoCA tests were able to show even mild cognitive impairment in these patients. We are compiling our full data for EEG but the early results show that the patients had less theta but had frequent bursts of alpha waves, a pattern seen commonly in patients with insomnia and neuropathic pain. They retain fewer items and take more time responding to items as compared to normal people. We need more studies to diagnose and treat mild cognitive impairment (“chemo brain”) in breast survivors as most of these patients are still working and can be a valuable part of the community.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-01

Title: Efficacy results of a phase 1b study of ONT-380, an oral HER2-specific inhibitor, in combination with capecitabine (C) and trastuzumab (T) in HER2+ metastatic breast cancer (MBC), including patients (pts) with brain metastases (mets)

Hamilton E, Borges V, Conlin A, Walker L and Moulder S. Sarah Cannon Research Institute, Nashville, TN; Tennessee Oncology, PLLC, Nashville, TN; University of Colorado Cancer Center, Aurora, CO; Providence Cancer Center, Eastside, Portland, OR; Cascadian Therapeutics, Inc., Seattle, WA and MD Anderson Cancer Center, Houston, TX.

Body: Background: ONT-380, a potent, highly selective, orally available small molecule inhibitor of HER2, has been associated with clinical benefit and minimal EGFR-type toxicities in single agent and combination studies of pts with HER2+ MBC, including pts with brain mets. In this Phase 1b study, ONT-380 was evaluated in combination with C and/or T in pts with HER2+ MBC who had been treated with T, a taxane, and ado-trastuzumab emtansine (T-DM1). During doublet dose escalation, ONT-380 300 mg PO BID was well tolerated and responses were seen in combination with either C or T alone; no maximum tolerated dose (MTD) was reached. ONT-380 300 mg PO BID was then studied in combination with both C and T as triplet therapy in order to provide dual blockade of HER2 in combination with cytotoxic chemotherapy. Safety and efficacy results of the triplet cohort are reported here.

Methods: ONT-380 300 mg PO BID was administered with C (1000 mg/m² PO BID 14 days of a 21-day cycle), and T (8 mg/kg IV loading; then 6 mg/kg IV once every 21 days). Prior treatment with T, a taxane, and T-DM1 were required; prior pertuzumab, lapatinib, or neratinib were permitted; prior capecitabine exposure was prohibited. Pts with asymptomatic brain mets (treated or untreated) were also eligible. Assessments included safety and tumor response by RECIST 1.1 and Modified CNS RECIST 1.1.

Results: Enrollment was complete as of December 2015. Interim safety and efficacy results as of May 27, 2016 are reported here, with mature data to be presented at the meeting. 27 pts were treated with ONT-380 + C + T: median age 50 y, 56% ER/PR+, 44% ER/PR, 47% PS 0, 53% PS 1. Pts received a median of 3 prior HER2 agents: 100% prior T and TDM-1, 74% prior pertuzumab, 37% prior lapatinib. 11 pts had brain mets at baseline, including 7 patients with untreated or progressive brain mets. Most toxicities were Gr 1. The most common adverse events (>40%) were diarrhea, nausea, palmar-plantar erythrodysesthesia (PPE), vomiting, and fatigue. Gr 3 events included PPE (11%), diarrhea (11%), fatigue (11%), and reversible increase in AST/ALT (7%). Dose reductions of ONT-380 were required in 6 pts, with 4 of these patients having continued disease control >6 months at the reduced dose. In 24 pts with measurable disease at baseline, ORR was 58%, with best responses of 1 CR, 13 PR, 6 SD, and 4 PD, and clinical benefit rate (SD ≥ 6 mos, PR, CR) in all 27 pts was 67%. Median PFS as of this data cut was 6.3 m (95% CI: 4.1-n/a). Median time on treatment for all 27 pts was 6.2 mo (range 1.4-21.4 mo), with 12 pts still active.

Conclusion: ONT-380 in combination with C and T exhibits an acceptable well tolerated safety profile and shows evidence of responses and long-term disease control in pts who have received contemporary standard of care treatment for HER2+ MBC with both pertuzumab and T-DM1, including pts with brain mets. Based on these encouraging data, a randomized placebo-controlled Phase 2 study (HER2CLIMB) is now enrolling to further evaluate the activity of ONT-380 in this population, including pts with brain mets.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-02

Title: Pertuzumab and trastuzumab plus standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer: Efficacy analysis of a phase II cardiac safety study (TRYPHAENA)

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Body: Background:

The multicenter, open-label Phase II TRYPHAENA study (NCT00976989) showed that neoadjuvant pertuzumab (P) + trastuzumab (H) + chemotherapy (anthracycline-containing or anthracycline-free) was generally well tolerated with low rates of symptomatic left ventricular systolic dysfunction (LVSD, the primary endpoint), in patients (pts) with HER2-positive, operable, locally advanced or inflammatory breast cancer. All three arms were highly clinically active: total pathologic complete response in the breast and axilla (tpCR; ypT0/is, ypN0) rates were 55–64%. We now report long-term disease-free survival (DFS), progression-free survival (PFS), overall survival (OS), and cardiac safety.

Methods:

Pts were randomized 1:1:1 to six 3-weekly neoadjuvant treatment cycles. Arm A: H + P (cycles 1–6) + fluorouracil, epirubicin, cyclophosphamide (FEC, cycles 1–3) + docetaxel (T) (cycles 4–6), Arm B: FEC (cycles 1–3) followed by T + H + P (cycles 4–6), Arm C: T + H + P + carboplatin (cycles 1–6). Adjuvant H was then given to complete 1 year of treatment. Doses: P 840mg loading and 420mg maintenance; H 8mg/kg loading and 6mg/kg maintenance; T 75mg/m², up to 100mg/m² if tolerated (Arms A and B); fluorouracil 500mg/m²; epirubicin 100mg/m²; cyclophosphamide 600mg/m²; carboplatin area under the plasma concentration–time curve 6. A preplanned descriptive analysis of DFS (time from surgery until disease progression or death), PFS (time from randomization until disease progression or death, equivalent to the common definition of event-free survival), and OS (time from randomization until death from any cause) was conducted 5 years after randomization of the last pt.

Results:

Median follow-up was balanced across arms (61.1 months in Arm A; 61.8 months in Arm B; 60.9 months in Arm C); 3-year Kaplan–Meier (KM) survival estimates and 95% CIs are shown in the table.

		Arm A n=73	Arm B n=75	Arm C n=77
3-year KM survival estimate, %	DFS	87 (79–95)	88 (80–96)	90 (82–97)
	PFS	89 (81–96)	89 (81–96)	87 (80–95)
	OS	94 (89–100)	94 (89–100)	93 (87–99)

For all three arms combined, the hazard ratio for DFS in pts who achieved tpCR versus those who did not achieve tpCR was 0.27 (95% CI 0.11–0.64). During post-treatment follow-up, 2/72 (2.8%) pts in Arm A, 3/75 (4.0%) in Arm B, and 4/76 (5.3%) in Arm C had LVSD (any grade). Of the pts with LVSD, only 1 pt experienced an event indicative of symptomatic LVSD (assessed as NYHA class II and grade ≥ 3). Also during this period, 8 pts in Arm A (11.1%), 12 (16.0%) in Arm B, and 9 (11.8%) in Arm C experienced LVEF declines to $<50\%$ and $\geq 10\%$ from baseline.

Conclusions:

The 3-year DFS and PFS rates were similar between treatment arms and were comparable to rates previously observed in the neoadjuvant NeoSphere study. Pts who achieved tpCR had improved DFS compared with those who did not achieve tpCR, supporting previous findings of an association between pCR and long-term outcomes (Cortazar *et al*, *Lancet* 2014). The

combination of P, H, and standard anthracycline-containing or anthracycline-free chemotherapy regimens was generally well tolerated and no new safety signals were identified with 5 years follow-up.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-03

Title: A randomized phase II study of Ki-67 response-guided preoperative chemotherapy for HER2-positive breast cancer

Takahashi M, Nishiyama Y, Hara F, Naito Y, Baba M, Sasaki M, Sato M, Watanabe K, Uemura Y, Yamaguchi T and Mukai H. NHO Hokkaido Cancer Center, Sapporo, Hokkaido, Japan; Kumamoto Shinto General Hospital, Kumamoto, Japan; NHO Shikoku Cancer Center, Matsuyama, Ehime, Japan; National Cancer Center Hospital East, Kashiwa, Chiba, Japan; University of Tokyo, Tokyo, Japan and Musashino Red Cross Hospital, Musashino, Tokyo, Japan.

Body: As for the HER2-positive breast cancer, there are many cases to be effective for neoadjuvant chemotherapy in comparison with other intrinsic subtypes. However, pCR is not provided by neoadjuvant chemotherapy in all cases. [Aim] This study evaluated the effectiveness of a therapeutic strategy that switches chemotherapy, based on Ki-67 tumor expression after initial therapy, relative to that of standard chemotherapy in patients with HER2-positive breast cancer. [patients and methods] Patients were randomly assigned to the control arm or the Ki-67 response-guided arm (Ki-67 arm). Primary tumor biopsies were obtained before treatment, and after three once-weekly doses of paclitaxel and trastuzumab to assess the interim Ki-67 index. In the control arm, paclitaxel and trastuzumab was continued for a total of 12 doses, regardless of the interim Ki-67 index. In the Ki-67 arm, subsequent treatment was based on the interim Ki-67 index. Early Ki-67 responders continued to received paclitaxel plus trastuzumab for a total of 12 doses, while early Ki-67 non-responders were switched to epirubicin plus cyclophosphamide every 3 weeks for three cycles with once-weekly trastuzumab for a total of 12 doses. The primary endpoint was the pathological complete response (pCR) rate. [Results] When 237 patients were enrolled, an interim analysis was conducted in 200 patients. There was almost linear correlation between the Ki-67 reduction rate at interim assessment and the pCR rate. The pCR rate in Ki-67 early non-responders in the Ki-67 arm (23.6%; 95% CI, 12.4 to 34.9) was inferior to that in the control arm (44.1%; 31.4 to 56.7; $p=0.025$). A strong correlation was not found between the Ki-67 reduction rate and the clinical response rate (Spearman's correlation coefficient 0.22).

pCR rate among Ki-67 early non-responders and responders

		Total	pCR		
		n	n	%	95%CI
Ki-67 early non responder	Control arm	59	26	44	31.4-56.7
	Ki-67 response guided arm	55	13	23	12.4-34.9
Ki-67 early responder	Control arm	21	10	47	26.3-69.0
	Ki-67 response guided arm	20	8	40	18.5-61.5

[Conclusions]The pCR rate in the Ki-67 arm was inferior to that in the control arm. A therapeutic strategy that switches chemotherapy, based on Ki-67 tumor expression after initial therapy, was not effective. The standard chemotherapy protocol remains as the recommended strategy for patients with HER2-positive breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-04

Title: Preliminary safety and efficacy of first-line pertuzumab combined with trastuzumab and taxane therapy for HER2-positive locally recurrent/metastatic breast cancer (PERUSE)

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Body: Background:

First-line (1L) docetaxel+trastuzumab+pertuzumab (THP) for HER2-positive metastatic breast cancer (MBC) significantly improved progression-free survival (PFS) and overall survival in the phase III CLEOPATRA trial, and led to the approval of this regimen. PERUSE (NCT01572038) was designed to assess the safety and efficacy of investigator's choice of taxane+HP for 1L locally recurrent (LR)/MBC, and allows exploration of safety and efficacy in a larger population.

Methods:

PERUSE is a multicenter, single-arm phase IIIb study. Patients (pts) with Eastern Cooperative Oncology Group performance status ≤ 2 and no prior systemic therapy for LR/MBC (except endocrine therapy) receive T, paclitaxel (PAC), or nab-PAC plus H (8 mg/kg \rightarrow 6 mg/kg every 3 weeks [q3w]) and P (840 mg \rightarrow 420 mg q3w) until disease progression (PD) or unacceptable toxicity. The primary endpoint is safety. Secondary endpoints include best overall response (BOR) and PFS.

Results:

The safety/ITT population includes 1436 pts at data cutoff (1 Apr 2016). Median follow-up was 17.2 mo (range, <1–41.4). The median pt age was 54 years (range 23–87), 64% had hormone receptor-positive disease, 27% received adjuvant H, and 75% had visceral disease. Pts received T, PAC, nab-PAC, H, and P for a median of 3.8 mo (range <1–24.2; n=791), 4.2 mo (<1–36.6; n=618), 3.9 mo (<1–17.3; n=73), 16.0 mo (<1–45.9; n=1435), and 16.1 mo (<1–45.9; n=1436), respectively.

Most pts discontinued taxanes for 'other' reasons (25%), adverse events (AEs; 16%), PD, or investigator decision (15% each); H, for PD or AEs (46% and 7%); and P, for PD or AEs (46% and 8%).

Grade ≥ 3 treatment-emergent AEs (TEAEs) of interest are shown in table 1. Serious TEAEs were reported in 282 (36%) pts on T, 185 (31%) on PAC, and 21 (32%) on nab-PAC. Preliminary efficacy by taxane is shown in table 2.

Table 1

Pts, n (%)	T	PAC	Nab-PAC
	n=775	n=589	n=65
Neutropenia	110 (14)	31 (5)	1 (2)
Diarrhea	62 (8)	50 (8)	4 (6)
Febrile neutropenia	81 (10)	7 (1)	0
Fatigue	21 (3)	10 (2)	1 (2)
Asthenia	16 (2)	10 (2)	0
Peripheral neuropathy	10 (1)	15 (3)	0
Anemia	11 (1)	12 (2)	2 (3)
Left ventricular dysfunction	1 (<1)	9 (2)	1 (2)

Table 2

	T	PAC	Nab-PAC
BOR, based on pts with measurable disease at baseline, n (%)	n=659	n=482	n=53
Complete	72 (11)	80 (17)	4 (8)
Partial	442 (67)	319 (66)	38 (72)
Stable disease	112 (17)	62 (13)	8 (15)
PD	18 (3)	12 (2)	2 (4)
Missing	15 (2)	9 (2)	1 (2)
PFS, ITT population	n=775	n=589	n=65
Pts with events, n (%)	439 (57)	324 (55)	40 (62)
Median, mo (95% confidence interval)	19.6 (17.4–22.6)	24.8 (20.7–27.0)	18.1 (11.9–34.2)
25th–75th percentile, mo	9.4–not reached (NR)	10.2–NR	7.9–NR

Conclusion:

The preliminary findings of this large, single-arm study suggest that safety and efficacy of 1L taxane+HP for HER2-positive LR/MBC are in keeping with the results of the phase III CLEOPATRA study. There was less febrile neutropenia with PAC/nab-PAC+HP compared with THP, and preliminary PFS was similar to that seen in the CLEOPATRA study for all taxane+HP combinations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-05

Title: Neoadjuvant non-pegylated liposomal doxorubicin plus paclitaxel, trastuzumab and pertuzumab in patients with HER2+ breast cancer – Final results of the SOLTI OPTI-HER HEART study

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Body: INTRODUCTION

Targeting HER2 by dual blockade with trastuzumab (T) and pertuzumab (P) in a taxane-based regimen is an active neoadjuvant treatment (NAT) of HER2+ early breast cancer (EBC). Addition of an anthracycline could further enhance this response, but potential cardiac toxicity is a concern. The Opti-HER HEART trial (NCT01669239) aims to optimize neoadjuvant treatment while minimizing cardiac risk, by combining T+P with a taxane and non-pegylated liposomal doxorubicin (NPLD).

MATERIAL AND METHODS

Phase II open-label, single-arm study of six 21-day cycles of NPLD (50mg/m² D1), paclitaxel (80mg/m² D1,8,15), T (4mg/kg C1D1, followed by 2mg/kg weekly), and P (840mg C1D1, followed by 420mg C2-6D1) as NAT for patients (pts) with stage II-III HER2+ BC. Primary objective was to evaluate cardiac safety of the combination, measured by the incidence of type A (symptomatic congestive heart failure) or type B [asymptomatic reduction of Left Ventricular Ejection Fraction (LVEF) value: ≥10% absolute decrease and LVEF<50%, LVEF<40% or any absolute decrease ≥20%] events, during NAT. Eighty-three pts were required to reject with 80% confidence the null hypothesis that the combination increases the incidence of cardiac events above the historical control of 18% (3% type A; 15% type B).

RESULTS

Between June 2013 and January 2015, 83 pts with HER2+ EBC (stage II 78%, stage III 22%) and adequate cardiac function (LVEF≥55%) were enrolled. Mean age was 50 years, N+ 47%, hormone receptor (HR) positive 71% and median baseline LVEF 66%. Eighty-five percent of pts completed 6 cycles of NAT, whereas 15% discontinued NAT due to toxicity. Adverse events (AEs) leading to dose adjustments/temporary interruptions and discontinuation of at least 1 drug occurred in 70% and 21% of pts, respectively. Primary objective was met with an incidence of cardiac events during NAT of 4% (95%CI 1-10, 3pts, all type B). Cardiac events until study completion (1 year) were 8% (all type B). All (but 2 cases with no follow-up data) were reversible and only 1 pt presented an asymptomatic LVEF<40%. Neutropenia (45%) was the most frequent hematological toxicity (G3/4 34%; febrile neutropenia 6%), less frequent in the 71% of pts that received primary G-CSF prophylaxis (G3/4 25% vs. 67%). Common non-hematological toxicities were diarrhea (74%; G3 7%), asthenia (78%; G3 11%) and neurotoxicity (52%; G3/4 10%). Pathological complete response (pCR) in breast+axilla (ypT0/is ypN0) was 60% (87% in HR-) and 69% in breast (91% in HR-).

	TOTAL	HR-	HR+
% ypT0/is (95% CI)	69 (58-79)	91 (72-99)	61 (47-74)
% ypT0/is ypN0 (95% CI)	60 (46-71)	87 (66-97)	50 (36-64)

CONCLUSIONS

The neoadjuvant combination of T+P, paclitaxel and NPLD does not increase the risk for cardiac events in HER2+ BC pts. Since cardiac toxicities may present later, long-term cardiac monitoring is essential. Efficacy in terms of pCR was remarkable, being higher to historical values of combinations with dual anti-HER2 blockade and one of the highest reported among HR-HER2+ BC. This regimen administered with primary G-CSF prophylaxis and cardiac function monitoring may be an effective and secure option for early and locally advanced HER2+ pts with good cardiac function.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-06

Title: Dual HER2-blockade with pertuzumab and trastuzumab in HER2-positive early breast cancer: A subanalysis of data from the randomized phase III GeparSepto trial

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Body: Background

Our recent randomized, multicenter phase III GeparSepto study (Untch M et al. Lancet Oncol 2016) found that substituting nab-paclitaxel for standard solvent-based paclitaxel significantly improved the pathologic complete response (pCR) rate in patients receiving a sequential regimen of taxane, epirubicin and cyclophosphamide as neoadjuvant treatment for high-risk primary breast cancer. Patients with HER2-positive tumors (32.8%; n=396) also received a combination of pertuzumab and trastuzumab: the present analysis focuses on efficacy and safety data from these HER2+ patients treated with the dual-blockade.

Methods

Patients with histologically confirmed early breast cancer (n = 1206) received either weekly paclitaxel 80mg/m² or weekly nab-paclitaxel 150/125mg/m², according to randomization), followed by four cycles of epirubicin 90 mg/m² plus cyclophosphamide 600 mg/m² q3w, with concurrent trastuzumab 6 mg/kg (loading (LD) dose 8 mg/kg) and pertuzumab 420 mg (LD 840 mg) q3w for those with HER2-positive tumors. The primary endpoint was pathologic complete response (pCR), defined as ypT0 ypN0.

Results

The GeparSEPTO trial included 396/1206 (32.8%) HER2+ primary breast cancer patients. 27.0% in the HER2-positive and 34.1% in the HER2-negative group had HR-negative disease. Baseline characteristics were otherwise comparable between HER2+ and HER2- patients. Higher rates of pCR were seen in HER2+, compared to HER2- tumors (57.8% vs 22.0%). The highest overall pCR rate was observed in the HER2+/HR- cohort with 71.0%; 66.7% with Pac and 74.6% with nab-Pac. In HER2+/HR+ pCR rate was 52.9% ; 49.4% with Pac and 56.4% with nab-Pac. Using the definition ypT0/is ypN0 for pCR; pCR rates were generally higher especially in the HER2+ cohort (66.2% (ypT0/is ypN0) vs 57.8% (ypT0 ypN0)) compared to 25.2% (ypT0/is ypN0) vs 22% (ypT0 ypN0)) in patients with HER2-negative tumors. The HER2+ patients experienced a significantly higher incidence of grade 3-4 adverse events 85.4% vs 78.0% in the HER2-cohort, p=0.003); grade 3-4 hematologic AEs 74.0% (HER2+) vs 69.5% (HER2-); p=0.120 with grade 3-4 anaemia 2.5% vs 0.9%; p=0.034); any grade thrombopenia 28.5% vs 21.8%; p=0.012) and febrile neutropenia 6.3 vs 3.3%; p=0.023. Any grade 3-4 non-haematological toxicities occurred in 38.4% vs 30.1%; p=0.005), with grade 3-4 diarrhea occurring in 7.6% vs 0.9%; p<0.001 of the patients. This had no impact on compliance. LVEF decreases from baseline were uncommon (7.6%) with 2.0% (HER2+) versus 0.4% (HER2-) of patients showing decreases to <50% along with a ≥10% decrease from baseline.

Conclusion

This is the largest cohort of patients with HER2-positive early breast cancer receiving a dual HER2-targeted neoadjuvant therapy of pertuzumab and trastuzumab, together with nab-paclitaxel or paclitaxel followed by epirubicin and cyclophosphamide. HER2+ patients experienced more noteworthy toxicity. The pCR rate were higher in the HER2+ cohort receiving the dual blockade and was highest in patients with in HER+/HR- particularly if nab-paclitaxel was substituted for paclitaxel.

The trial is financially supported by Celgene and Roche.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-07

Title: Preliminary safety and efficacy of first-line pertuzumab combined with trastuzumab and taxane therapy in patients ≥ 65 years with HER2-positive locally recurrent/metastatic breast cancer: Subgroup analyses of the PERUSE study

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Body: Background:

First-line (1L) docetaxel+trastuzumab+pertuzumab (THP) is approved for HER2-positive metastatic breast cancer (MBC), based on superior progression-free (PFS) and overall survival vs TH in the phase III CLEOPATRA trial. Predefined CLEOPATRA subgroup analyses suggested that THP use should not be limited by patient (pt) age: toxicity was increased but efficacy was similar in pts ≥ 65 vs < 65 years. PERUSE (NCT01572038) examined investigator's choice of taxane+HP for HER2-positive locally recurrent (LR)/MBC. We report preliminary safety and efficacy in pts ≥ 65 and < 65 years.

Methods:

In this multicentre, single-arm phase IIIb study, pts with ECOG PS ≤ 2 and no prior systemic therapy for LR/MBC (except endocrine therapy) receive investigator's choice of T, paclitaxel (PAC), or nab-PAC + H (8 \rightarrow 6 mg/kg q3w) + P (840 \rightarrow 420 mg q3w) until disease progression/unacceptable toxicity. The primary endpoint is safety. Secondary endpoints include PFS, estimated using the Kaplan–Meier method.

Results:

At data cutoff (1 Apr 2016), 1436 pts were included in the safety/ITT populations. 312 pts (22%) were aged ≥ 65 at study entry; they were more likely to have comorbidities than the < 65 group (91% vs 69%, including vascular disorders [56% vs 22%]; most commonly hypertension [51% vs 17%]) and be ECOG PS 1–2 (50% vs 38%).

Proportions of pts with visceral and hormone receptor-positive disease were similar (78% vs 74% and 65% vs 64%, respectively). Median duration of chemotherapy was similar but median exposure to antibodies was shorter in the ≥ 65 vs < 65 group: H 11.7 mo (range < 1 –45.8) vs 17.5 mo (< 1 –45.9); P 11.6 mo (< 1 –45.8) vs 17.8 mo (< 1 –45.9).

Interruptions/discontinuations due to treatment-emergent adverse events (TEAEs) were more common in pts ≥ 65 compared with pts < 65 with respect to taxanes (50% vs 35%), H (41% vs 26%), and P (38% vs 23%).

TEAEs related to taxanes, H, and P were generally similar in pts aged ≥ 65 and < 65 (93% each, 67% vs 62%, and 71% vs 70%, respectively).

Serious TEAE rates and most common grade ≥ 3 TEAEs of interest are shown in table 1.

Table 1

Pts, n (%)	<65			≥ 65		
	T	PAC	Nab-PAC	T	PAC	Nab-PAC
	n=638	n=431	n=51	n=137	n=158	n=14
Serious	223 (35)	124 (29)	18 (35)	59 (43)	61 (39)	3 (21)
Grade ≥ 3						
Neutropenia	88 (14)	18 (4)	1 (2)	22 (16)	13 (8)	0
Diarrhea	45 (7)	33 (8)	3 (6)	17 (12)	17 (11)	1 (7)
Febrile neutropenia	67 (11)	5 (1)	0	14 (10)	2 (1)	0

Fatigue	19 (3)	3 (1)	1 (2)	2 (1)	7 (4)	0
Asthenia	9 (1)	2 (<1)	0	7 (5)	8 (5)	0
Peripheral neuropathy	8 (1)	8 (2)	0	2 (1)	7 (4)	0
Anemia	9 (1)	4 (1)	1 (2)	2 (1)	8 (5)	1 (7)
Left ventricular dysfunction	0	5 (1)	0	1 (1)	4 (3)	1 (7)

Preliminary median PFS at the time of this analysis was 23.2 mo in the <65 group (95% CI 20.7–25.3; median follow-up 18 mo) and 14.7 mo in the ≥65 group (95% CI 12.8–20.2; median follow-up 14 mo); 511/1124 (45%) and 116/312 (37%) pts were censored, respectively.

Conclusion:

Preliminary findings with 1L taxane+HP in this large cohort of LR/MBC pts show that pts aged ≥65 years had more comorbidities and poorer PS at study entry compared with those aged <65, and that serious TEAEs were noted more frequently. Treatment exposure and PFS were shorter for pts aged ≥65.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-08

Title: A phase I/II of S-222611, a reversible EGFR and HER2 inhibitor, combined with trastuzumab +/- chemotherapy in patients with HER2-positive metastatic breast cancer

Rafii S, Macpherson I, Baird R, Saggese M, Spiliopoulou P, Kumar S, Italiano A, Bonnetterre J, Campone M, Cresti N, Posner J, Takeda Y, Arimura A and Spicer J. Sarah Cannon Research Institute UK, London, United Kingdom; Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom; Cambridge Cancer Centre, Cambridge, United Kingdom; Institute Bergonie, Bordeaux, France; Centre Oscar Lambert, Lille, France; Institut de Cancérologie de l'Ouest, Nantes, France; Northern Centre for Cancer Care, The Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle, United Kingdom; Shionogi & Co. Ltd., Osaka, Japan and King's College London, Guy's Hospital, London, United Kingdom.

Body: Background: S-222611, an oral, reversible EGFR and HER2 inhibitor, has been shown to be well-tolerated as monotherapy at a dose of 800mg daily with good anti-tumor activity in patients previously treated with other anti-HER2-based regimens. This study evaluated the tolerability and safety of daily oral administration of S-222611 (S) in combination with trastuzumab (T), trastuzumab + vinorelbine (T+V) and trastuzumab + capecitabine (T+C) in HER2-positive (HER2+) metastatic breast cancer (MBC) with or without brain metastases.

Methods: This study was performed as a 3+3 dose escalation followed by expansion to examine the tolerability and safety of S in combination with T, T+V and T+C in Arms A, B and C, respectively. S was administered orally once daily, starting at a dose of 400mg in Arm A, and 200mg in Arms B and C. The dosing of T was 8mg/kg loading followed by 6mg/kg or fixed dose of 600mg subcutaneously every 21 days as recommended. V was administered at 60mg/m² orally on Day 1 and 8 of a 21-day cycle, and C 1000mg/m² orally daily for 14 days followed by a 7-day rest period. All patients had HER2+ MBC and were required to have progressed following at least one prior line of anti-HER2 therapy. Prior treatments with V and C were permitted. Anti-diarrhea prophylaxis with loperamide was not required.

Results: A total of 45 patients were enrolled. All patients had received prior anti-tumor regimens including T (n=45), T-DM1 (n=26), pertuzumab (n=9) and lapatinib (n=12). The clinically recommended doses of S at which most adverse events were manageable, were determined as: 600mg in Arm A, 200mg in Arm B and 400mg in Arm C. Dose limiting toxicities included Grade 3 diarrhea for Arm A; and Grade 4 neutropenia, Grade 3 Hypokalemia and Hypophosphatemia for Arm B. As of 13 May 2016, treatment is ongoing in 2 patients. No other Grade 4 AEs related to S-222611 have been observed. Grade 3 bilirubin elevation was observed in 5/45 patients, probably due to transporter (UGT1A1) inhibition, while no G3/4 liver dysfunction was reported. RECIST partial responses (PR) were observed in 6 of 9 patients in Arm A and 5 of 9 patients in Arm C, at respective clinically recommended doses. Nine of 45 patients had brain metastases; 4 of these patients showed RECIST PR including an intracranial tumor response in one patient (400mg in Arm C) who had prior treatments with paclitaxel, T+C, T-DM1 and V after diagnosis of BM.

Conclusions: The clinically recommended doses of S-222611 combined with T, T+V and T+C were determined for further clinical studies. Clinical benefit (PR and SD ≥6 month) was seen with each combination even in heavily pre-treated HER2+ MBC patients.

Summary of the safety and efficacy of S-222611 (S) combination.

	Dose (mg)	n	DLT (1st cycle)	G3 Diarrhea during study (N of patients)	RECIST tumor response, PR n/SD ≥6M n	ORR n (%)	CBR n (%)
Arm A: S + T	400	5	0	1	0/1	0/5 (0%)	1/5 (20%)
Arm A: S + T	600	9	0	3	6/0	6/9 (67%)	6/9 (67%)
Arm A: S + T	800	7	1	4	1/1	1/7 (14%)	2/7 (29%)
Arm B: S + T + V	200	5	0	2	0/4	0/5 (0%)	4/5 (80%)
	400	2	2	1	1/0	1/2 (50%)	1/2 (50%)

Arm B: S + T + V						
Arm C: S + T + C	200	4 0	1	0/1	0/4 (0%)	1/4 (25%)
Arm C: S + T + C	400	9 0	2	5/0	5/9 (56%)	5/9 (56%)
Arm C: S + T + C	600	4 0	2	2/1	2/4 (50%)	3/4 (75%)

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-09

Title: Cardiac biomarkers for early detection and prediction of trastuzumab and/or lapatinib-induced cardiotoxicity in patients with HER2 positive early breast cancer (BIG 1-06)

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Body: Background: Anti-HER2 agents are associated with an increased risk of cardiotoxicity, occurring mostly during treatment and being generally reversible. To date, no cardiac biomarkers have been validated as predictive markers of cardiotoxicity in patients (pts) receiving anti-HER2 therapies. We evaluated high sensitivity (HS) troponin T (TnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) in pts treated with neoadjuvant anti-HER2 therapies to evaluate if early elevation of cardiac biomarkers predicts early or late cardiac events.

Material and Methods: 455 pts were randomized to receive either lapatinib (154 pts), trastuzumab weekly (149 pts), or lapatinib with trastuzumab for a total of 6 weeks (152 pts). After this biological window, pts continued on the same targeted therapy plus 12-weekly paclitaxel until definitive surgery. After surgery, patients received adjuvant FEC (3x) followed by the same HER2 therapy as in the neoadjuvant phase for further 34 weeks (to complete 52 weeks in total). Eligible pts had an LVEF \geq 50%. Cardiac biomarkers were centrally tested in plasma samples collected at baseline, week 2 (W2) and at surgery. Elevated biomarker was defined as either TNT >0.015 μ g/L or NT-proBNP >125 pg/mL. The small number of pts and rarity of marker elevations allowed only descriptive analyses.

Results: At a median follow-up of 3.8 years, 280 pts had cardiac biomarkers tested at baseline (61.5%) and NT-proBNP was elevated in 39 pts (13.9%) while TNT was elevated in only 1 pt (0.35%). From those 39 pts with NT-proBNP elevated at baseline, only 1 pt experienced a cardiac event. Also, hypertension was presented in 14 pts (24.5%) with elevated NT-proBNP. Serial measurements were available in 173 pts (61.7%) and NT-proBNP was elevated in 25, 10 and 21 pts at baseline, W2 and at surgery, respectively, while among 171 pts (61%) TNT was elevated in 1, 1 and 5 pts, respectively. There were 13 cardiac events in 11 pts in the entire trial. Five of these pts had a measurement of cardiac biomarkers at baseline (non-elevated NT-prBNP and TNT were observed in 4 and 5 pts), 9 of them had a measurement at week 2 (non-elevated NT-prBNP and TNT were observed in 8 and 9 pts) and 3 of them at surgery (non-elevated NT-prBNP and TNT were observed in 3 and 3 pts), respectively. The following table show the low specificity of the cardiac biomarkers.

	Patients without cardiac event (N)	Patients with cardiac event (N)	Patients with elevated marker
Elevated NT-proBNP			
Baseline	38	1	39
Week 2	29	1	30
Surgery	40	0	40
Elevated TNT			
Baseline	1	0	1
Week 2	1	0	1
Surgery	6	0	6

During adjuvant phase, 13 pts experienced LVEF drop to <50% and none had either NT-proBNP elevated or TNT at surgery.

Conclusions: Despite the small sample size, we demonstrated that cardiac biomarkers are rarely elevated in pts receiving anti-HER2 therapies, which is probably due to the absence of myocyte necrosis with respect to TNT, and the lack of chamber dilation in the case of NT-proBNP after exposure to these drugs. In addition, few cardiac events were present in this population not previously exposed to anthracycline-based chemotherapy; therefore the use of cardiac biomarkers in this population may be unnecessary.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-10

Title: Characterization of neratinib-induced diarrhea in patients with early-stage HER2+ breast cancer: Analyses from the phase III ExteNET trial

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Body: Background: ExteNET is an ongoing randomized placebo-controlled phase III trial designed to investigate the efficacy and safety of 12 months' treatment with neratinib in women with stage I–III HER2+ breast cancer after completion of adjuvant trastuzumab. At 2 years after randomization, a significant improvement in invasive disease-free survival was evident with neratinib vs placebo (stratified hazard ratio 0.67, 95% CI 0.50–0.91; P=0.0091) [Chan et al. *Lancet Oncol* 2016]. Neratinib was generally well tolerated with a low incidence of grade 3/4 events; diarrhea was the most common adverse event (grade 3, 39.8%, grade 4, 0.1%). We reviewed the safety data related to this study in order to better characterize the occurrence of neratinib-induced diarrhea (NID) and its impact on health-related quality of life (HRQoL).

Methods: Patients received neratinib 240 mg once daily or placebo for 12 months. Antidiarrheal prophylaxis was not dictated by the protocol, but investigators were advised to treat diarrhea early. Adverse events were monitored until 28 days after the last dose of study drug, and graded according to NCI-CTCAE, v3.0. Patient-reported HRQoL questionnaires (Functional Assessment of Cancer Therapy–Breast [FACT-B], v4; EuroQol 5-Dimensions [EQ-5D]) were completed at baseline and 1, 3, 6, 9 and 12 months. Descriptive analyses of FACT-B and EQ-5D scores by maximum grade of diarrhea were performed in patients with post-baseline HRQoL assessments. ClinicalTrials.gov: NCT00878709.

Results: Of 2840 women randomized, 2816 (1408 per group) received at least one dose of study drug and were evaluable for toxicity. Diarrhea was more common with neratinib than placebo (all-grade: 95.4% vs 35.4%; grade 3: 39.8% vs 1.6%; grade 4: 0.1% vs 0%). Median time to onset of any grade NID was 2 (IQR 2–4) days. Most grade 3 NID occurred early in the course of treatment with a reduction in frequency thereafter; 28.6% of patients had grade 3 events during month 1 decreasing to approx. 6% or less after month 3. For those experiencing grade 3/4 NID, the median number of events was 2 (IQR 1–3) with a median cumulative duration of 5 (IQR 2–9) days. NID resulted in dose reductions in 26.4%, dose holds in 33.9%, drug discontinuation in 16.8%, and hospitalizations in 1.4% of patients. In the neratinib group, FACT-B and EQ-5D scores decreased with increasing severity of diarrhea (Table); the changes in scores were not considered to be clinically meaningful.

Instrument	MID range	n	Mean score ± SD		
			No diarrhea	Grade 1/2	Grade 3/4
FACT-B total	7–8	1269	112.7 ± 18.1	110.6 ± 17.3	107.6 ± 19.0
EQ-5D index	0.09–0.1	1273	0.86 ± 0.17	0.84 ± 0.18	0.82 ± 0.19
EQ-5D VAS	7–10	1273	79.6 ± 14.1	78.6 ± 14.6	75.8 ± 15.6

MID, minimally important difference; SD, standard deviation; VAS, visual analogue scale.

Conclusion: NID has a distinct clinical pattern, with a high incidence of grade 3 events in the first month and a dramatic decrease thereafter. In the absence of antidiarrheal prophylaxis, patients with severe diarrhea experience a median of 2 events lasting for a cumulative duration of 5 days. Loperamide prophylaxis given early in the course of treatment is currently being investigated in the prevention of NID with promising early results.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-11

Title: T-DM1 in HER2 positive advanced breast cancer patients: Real world practice from a multicenter observational study

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Body: Background: T-DM1 showed remarkable activity in metastatic HER-2 positive breast cancer (mBC) and it was recently approved for clinical use in patients (pts) who previously failed Trastuzumab- and Taxanes-based therapies. Currently, little is known on the performance of T-DM1 in a "real life" scenario. Therefore, we investigated effectiveness and safety of T-DM1 in Italian daily practice.

Methods: Pts baseline characteristics and clinical outcome of pts with HER-2 positive mBC treated with T-DM1 between 2013 and 2015 at 20 Italian Institutions were retrospectively collected and analyzed.

Results: 300 pts were included in our analysis. Median age was 51 years (27-78); visceral metastases were present in 204 (68%) pts and brain metastases in 86 (29%). It is noteworthy that 111 (37%) pts received T-DM1 as pure second line, 83 (28%) as third line and 96 (32%) as further lines. Moreover 10 (3%) pts had T-DM1 as first line because disease recurrence occurred during or adjuvant trastuzumab of within 6 months of its completion. The overall response rate (ORR) was 40%, global disease control rate (gDCR) 64%, median progression-free survival (PFS) 7.0 months (C.I.95%: 5.6-8.4) and overall survival (OS) at 2 years 63%. Pts with 1, 2 and 3 or more metastatic site had OS at 2 years of 87%, 67% and 46%, respectively ($p < 0.0001$). When T-DM1 was given as second line the PFS was 8.0 months and beyond second-line was 6.8 months. Interestingly, for 38 (13%) pts who progressed after Pertuzumab-plus trastuzumab and taxanes as first line treatment, ORR and gDCR were similar to pertuzumab-naïve patients (38% and 62%, respectively) However PFS was 5.0 months (C.I.95%: 4.3-5.7) compared to 9.0 (95% C.I. 5.5-12.4) achieved in pts not receiving a previous pertuzumab-based treatment. Most frequent grade ≥ 3 toxicities were thrombocytopenia (2.6%), alopecia (2.1%), hypertransaminasemia (2.2%), neutropenia (1.3%), asthenia (1.3%) and diarrhea (0.4%).

Conclusions: To our knowledge, this is the first real life, multicenter retrospective analysis evaluating efficacy and safety of T-DM1 in pretreated HER-2 positive mBC pts. We observed remarkable results in terms of PFS and OS, especially when T-DM1 was given early in the course of metastatic disease. Shortened PFS in patients progressing after pertuzumab suggest further analyses to better define possible molecular mechanisms of cross-resistences between two molecules. As a whole there was no evidence of significant or unexpected toxicities. Although these findings should be taken with caution due to the retrospective analysis and the different lines of previous treatment considered, we confirmed the potential therapeutic role of T-DM1 across a heterogeneous population of HER-2 positive mBC patients. The final analysis will be presented to the meeting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-12

Title: Preference of trastuzumab administration route (intravenous or subcutaneous) in patients in the Czech Republic. Cross-sectional study on 429 patients

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Body: Background: Trastuzumab is the treatment for HER2-positive breast cancer in the metastatic, adjuvant, and neoadjuvant settings. Trastuzumab is available in intravenous (i.v.) and subcutaneous (s.c.) application form. PrefHer study published in 2013 described patients' preference for use of subcutaneous trastuzumab. Our aim was to find out and describe if any preference exists in real clinical practice in the Czech Republic.

Objectives: To analyse reasons for preference of s.c. or i.v. application of trastuzumab in patients with HER2-positive breast cancer in real clinical setting in the Czech Republic.

Methods: A questionnaire based data collection from patients treated in Comprehensive Cancer Centres between January 2015 and July 2015.

Results: We received 429 questionnaires from patients. Data analysis has been conducted in 301 (70.2%) patients' questionnaires after quality control for questionnaires completeness. Out of the 301 patients there were 151 who had ≥ 11 i.v. applications, 137 patients with 3-10 i.v. applications and 13 patients with only 2 i.v. applications. Majority of patients were treated in adjuvant setting (62.8%, n=189), 21.9% (n=66) received neoadjuvant treatment and 15.3% (n=46) patients were treated for advanced disease. Only 33 patients had experience with ≥ 11 s.c. applications, 222 patients with 3-10 s.c. applications and 46 patients with 2 s.c. applications. Subcutaneous form was given in 74.8% (n=225) in adjuvant setting, in 20.3% (n=61) in advanced setting and in 5.0% (n=15) in neoadjuvant setting. Pain related to trastuzumab application was assessed – 52.2% (n=157) patients stated that s.c. application is less painful, 34.2% (n=103) did not see difference and 13.6% (n=41) patients felt that i.v. form is less painful. When assessing site reactions majority (62.4%, n=184) of patients did not see difference between application forms, 20.0% (n=59) patients preferred s.c. form and 17.6% (n=52) preferred i.v. form. Lower anxiety related to trastuzumab application was reported with s.c. form (46.0%, n=137). Only 6.0% (n=18) patients reported lower anxiety with i.v. application and 48.0% (n=143) did not see any difference. Vast majority (92.7%, n=279) patients described s.c. form as more comfortable, 4.7% (n=14) patients did not see difference and only 2.7% (n=8) described i.v. form as more comfortable. Overall 95.0% (n=286) patients preferred s.c. application form of trastuzumab. The main reasons are time savings (86.7%, n=261) and better comfort (71.8%, n=216). Only 10 patients (3.3%) favoured i.v. application form. The main reason for i.v. preference is lower pain and lower incidence of complications (2.0%, n=6). The remaining 5 patients (1.7%) had no preference of any application form.

Conclusion: Patients treated in Comprehensive Cancer Centres in the Czech Republic prefer subcutaneous application form of trastuzumab. The main reasons for their preference are time savings and better application comfort.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-13

Title: Comparative toxicity and effectiveness of trastuzumab combined with anthracycline versus platinum chemotherapy regimens for adjuvant therapy of HER2+ breast cancer in older women

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Body: Rationale: The combination of chemotherapy and the monoclonal antibody trastuzumab is the standard of care for systemic adjuvant therapy of HER2-positive breast cancer, substantially improving patient outcomes. Two regimens have been widely adopted for use in the United States, adriamycin/cyclophosphamide/paclitaxel/trastuzumab (ACTH) and docetaxel/carboplatin/trastuzumab (TCH). While other options are available in the neoadjuvant and low-risk settings, ACTH or TCH remain standard of care for many women with early-stage HER2+ disease. No head-to-head comparison of these regimens has been conducted in a clinical trial, and the clinical trial data have limited generalizability due to the exclusion of older women and the low representation of minorities and those with significant co-morbidities.

Research Objectives: We used SEER-Medicare data from 2005-2013 to conduct a comparative effectiveness study of ACTH vs TCH among patients over 65 receiving trastuzumab-based adjuvant chemotherapy. An intent-to-treat analytic design was applied and propensity score matching was used to account for selection bias and to balance cohort characteristics between treatment arms. Outcomes included toxicity-related hospitalization, survival, and regimen completion (receiving ≥ 270 days of trastuzumab). Toxicity was evaluated using inpatient and emergency room claims for chemotherapy-related adverse events in the first six months of therapy, as well as all-cause hospitalization over the same period. Data from 1077 patients with HER2+ disease were analyzed and the propensity-matched sub-sample included 416 women.

Results: Over time there was a significant shift in regimen use between ACTH vs TCH, with 88% of patients in 2005 receiving ACTH compared to only 15% by 2011. Using the propensity score-matched patients, we found no difference between regimens in healthcare utilization for chemotherapy-related adverse events or all cause hospitalization. (RR=0.99, 95% CI 0.64-1.51) Patients receiving TCH were significantly more likely to complete trastuzumab (89% for TCH vs 77% for ACTH). There was no difference between regimens in five year breast cancer-specific survival or overall survival. Results of a sensitivity analysis limited to patients who completed trastuzumab were similar to the primary analysis.

Conclusions: Among older women with HER2+ breast cancer receiving multi-agent regimens with similar levels of medical comorbidity, ACTH compared to TCH did not appear to be associated with a higher rate of serious adverse events or hospitalizations, but was associated with a lower rate of completion of adjuvant trastuzumab. Ability to evaluate cardiotoxicity in this claims-based analysis was limited. We did not detect a difference in five year survival outcomes for ACTH compared to TCH. In the context of limited evidence in older patients, there appears to be little difference between the two regimens in terms of either toxicity or efficacy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-14

Title: A randomized phase II trial of trastuzumab + capecitabine versus lapatinib + capecitabine in patients with HER2-positive metastatic breast cancer previously treated with trastuzumab and taxanes: WJOG6110B/ELTOP

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Body: Background: In patients with HER2-positive metastatic breast cancer (MBC) who progressed on trastuzumab (H)-based therapy, both continuing H beyond progression and switching to lapatinib (L) in combination with chemotherapy are valid options. However, it is unclear which strategy is more effective and how we can select a proper strategy in each patient.

Methods: We conducted an open label, multicenter, randomized phase II trial to comparatively evaluate efficacy and safety of H + capecitabine (X) (HX) or L + X (LX) in women with HER2-positive MBC who were previously treated with taxanes and progressed on H-containing regimens. Patients treated with more than two chemotherapy regimens for MBC were excluded. Those treated with pertuzumab and/or T-DM1 were allowed to enroll in this study. Patients with brain metastases were also included if they are asymptomatic. Patients received H (4mg/kg loading then 2mg/kg weekly or 8mg/kg loading then 6mg/kg every 3 weeks) and X (2500 mg/m²/day on days 1-14 every 3weeks) in HX arm and L (1250 mg/day) and X (2000 mg/m²/day on days 1-14 every 3weeks) in LX arm until progression or intolerable toxicity. The primary endpoint was progression-free survival (PFS) and secondary endpoints included overall survival (OS), objective response rate (ORR), proportion of subjects progressing with brain metastases as site of first progression, and safety. We also assessed biomarkers in tumor tissues and circulating cell-free DNA.

Results: Between May 2011 and December 2014, 86 patients (43 in HX arm and 43 in LX arm) were enrolled in this study. Median age was 58 years (range 34-81), ECOG performance status was 0 (63%), 1 (35%), or 2 (2%), 63% had hormone receptor-positive disease, 15% had brain metastases, 56% had relapsed after primary surgery, and 23% had received adjuvant or neo-adjuvant trastuzumab. Median follow-up time was 44.6 months. Median PFS was 6.1 months in HX arm and 7.1 months in LX arm (hazard ratio 0.81 90% CI 0.55-1.21; p=0.39), median OS was 31.0 months in HX arm and not reached in LX arm (hazard ratio 0.58 95% CI 0.26-1.31; p=0.18), ORR was 40% in HX arm and 41% in LX arm (p=1.00), disease control rate was 73% in HX arm and 92% in LX arm (p=0.038), and proportion of subjects progressing with brain metastases as site of first progression was 5% in HX arm and 5% in LX arm. Grade 3-4 toxicities included hand-foot syndrome (21% in HX arm and 21% in LX arm) and diarrhea (9% in HX arm and 16% in LX arm). In subgroup analyses, PFS benefit in LX arm compared to HX arm was significantly larger among patients who had received previous systemic treatment for metastatic disease for less than 1 year (interaction p=0.007). Subgroup analyses by biomarkers will be presented at the meeting.

Conclusions: In women with HER2-positive MBC previously treated with trastuzumab and taxanes, lapatinib + capecitabine tended to yield better PFS and OS than trastuzumab beyond progression + capecitabine, although they were not statistically significant.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-15

Title: Trastuzumab IV *versus* SC: A time, motion and cost assessment in a lean operating day care oncology unit

Tjalma WAA AA, Van den Mooter T, Mertens T, Bastiaens V, Altintas S, Huizing MT T, Trinh B, Van Dam P, Peeters M and Papadimitriou K. University Hospital Antwerp - University of Antwerp, Edegem, Antwerpen, Belgium.

Body: Purpose:

Primary endpoint: quantification of the active healthcare professional (HCP) time associated with the standard intravenous (IV) infusion of trastuzumab (Herceptin®) compared to the subcutaneous (SC) administration in the treatment of patients with HER2-positive early breast cancer.

Secondary endpoints: quantification of the preparation and administration related costs (HCP time, consumables and drug wastage), patient chair time and the total time spent in the day care unit for both routes of administration.

Patients and Methods:

A local observational project was conducted in the LEAN Operating Day Care Oncology Unit of the Antwerp University Hospital (Belgian registration number: B300201525036).

Independent observers measured the duration of each trastuzumab IV and SC related task that HCPs undertook and recorded patient time in the chair and the day care unit. The type and quantity of medical consumables used with each route were also registered. A total of 105 patient episodes were recorded (40 IV, 65 SC) from October 2015 until May 2016.

The recorded active HCP time corresponded to the active preparation and administration time of the medication. Total preparation and administration time was calculated as the mean sum of task times, both for IV and SC formulations. The cost of each route of administration was calculated as the mean cost of HCP time plus the mean cost of consumables used. Drug wastage that occurred with the IV formulation was also taken into account. The total active HCP time was quantified by the Hospital's Human Resources department. Consumables were quantified using hospital pharmacy data and online sources. Patient chair time was measured as the actual time spent on the chair. The total time spent in the day care unit was calculated as the time between the patient's registry and the time the patient left the hospital.

Results: The mean active HCP time for preparation and administration of IV was 64 minutes compared to 14 minutes for SC. The mean cost for preparation and administration was €303,12 for IV (€33,8 of HCP time, €23,56 of consumables and €245,76 of drug wastage) versus €10,39 for SC (€7,68 of HCP time and €2,71 of consumables). Mean patient chair time and the total time spent in the day care unit for IV was 133 and 165 minutes respectively, and 11 and 50 minutes for SC.

Time, Motion and Cost assessment of Trastuzumab IV versus SC

Mean administration route per cycle	IV	SC
Active HCP time (minutes)	64	14
Cost HCP time (€)	33,8	7,68
Cost consumables (€)	23,56	2,71
Drug wastage (€)	245,76	0
Patient chair time (minutes)	133	11
Total time spent in the hospital (minutes)	165	50

SC administration of trastuzumab resulted in a HCP time saving of 50 minutes (versus IV) with a total cost saving of €292,73 per administration. This leads to a potential saving of €5.269,16 over a full course of adjuvant treatment (18 cycles).

Conclusion: Substituting IV with SC administration of trastuzumab leads to a substantial reduction in active HCP time. As a

result, the overall costs consisting of HCP time, consumables and drug wastage are also reduced. Additionally, the reduced patient chair and unit time could provide increased capacity within existing resources in a LEAN Operating Day Care Oncology Unit.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-16

Title: Phase III trial to evaluate patient's preference for subcutaneous versus intravenous trastuzumab administration in patients with HER2 positive advanced breast cancer (ABC) under IV trastuzumab (IV-t) treatment for at least 4 months. ChangHER-SC study (GEICAM/2012-07)

Ciruelos EM M, Montañó Á, Rodríguez CA A, González-Flores E, Lluch A, Garrigós L, Quiroga V, Antón A, Malón D, Chacón JI I, Velasco M, Gonzalez-Cortijo L, Jolis L, Pascual T, Amigo Y, Casas M, Cámara MC, Carrasco E and Casas A. Hospital Universitario 12 de Octubre, Madrid, Spain; Hospital Universitario Virgen del Rocío, Sevilla, Spain; Hospital Universitario de Salamanca-IBSAL, Zaragoza, Spain; Hospital Universitario Virgen de las Nieves, Granada, Spain; Hospital Clínico de Valencia, Instituto de Investigación Sanitaria INCLIVA, Universitat de València, Valencia, Spain; Hospital del Mar, Barcelona, Spain; Institut Català d'Oncologia Badalona-HU Germans Trias i Pujol, Badalona, Barcelona, Spain; Hospital Universitario Miguel Servet, Zaragoza, Spain; Hospital Universitario de Fuenlabrada, Madrid, Spain; Hospital Virgen de la Salud, Toledo, Spain; Hospital de Mataró (Consorci Sanitari del Maresme), Mataró, Barcelona, Spain; Hospital Universitario Quirón de Madrid, Madrid, Spain; Hospital General de Granollers, Granollers, Barcelona, Spain; GEICAM, Spanish Breast Cancer Group, Madrid, Spain and Hospital Universitario Virgen del Rocío, Sevilla, Spain.

Body: Background: Patients with HER2-positive ABC, receive anti-HER2 treatment for several months or even years. IV-t is administered weekly or 3-weekly, mandating patients to visit the hospital on a regular basis to receive infusions. This has inconveniences for patients and increase treatment costs. Subcutaneous administration could improve convenience of trastuzumab therapy. This study was designed to evaluate patient's preference for IV-t or SC trastuzumab (SC-t) in ABC patients.

Methods: This is a phase III, open label, multicenter study in patients with HER2 positive ABC, receiving IV-t for at least 4 months and without evidence of disease progression. Patients received 600 mg of SC-t, either from a vial or from a single injection device (SID), every 3 weeks for 4 cycles. Before starting SC-t, patients received an additional IV-t cycle. Patients were randomized 1:1 to arm A, receiving 2 cycles of SC-t with vial followed by 2 cycles with SID or arm B, receiving the opposite sequence. After cycle 4, patients decided to continue with IV-t or SC-t till disease progression. Stratification criteria were the associated therapy (Chemotherapy, Hormone-therapy or none) and its administration route (IV, oral or none). Patients completed a questionnaire of experiences and preferences at three time points: before starting SC-t, after cycle 2 and after cycle 4. Health Care Professionals completed a satisfaction questionnaire every 5 patients. The primary objective was to evaluate patient's preference for IV-t or SC-t (after cycle 2) and, secondary objectives were, to evaluate patient's preference between the two SC-t administrations (vial or SID) after cycle 4, Health Care Professional satisfaction, associated costs of the administration options (Time and Motion pharmacoeconomic study) and safety.

Results: From September-13 to July-15, 166 patients were randomized (81 arm A, 85 arm B) in 26 Spanish sites from GEICAM. Median age was 60 years (35-93), 88% of patients were postmenopausal and 123 and 42 had an ECOG PS of 0 and 1, respectively. The median duration of prior IV-t for ABC was 1.8 years (range: 0.3-14). Patients received a median of 2 previous lines of Chemotherapy and/or Hormone-therapy (range: 1-9). Twenty patients were receiving pertuzumab at inclusion. According to patient questionnaires completed after cycle 2, 137 patients preferred the SC-t (66 arm A, 71 arm B), 11 the IV-t (6 arm A, 5 arm B), 11 didn't have a preference (4 arm A, 7 arm B), and 7 didn't answer (3 progressed, 2 withdrew participation before cycle 2 and 2 unknown reason). Three of the 11 patients choosing IV-t were receiving IV treatment as accompanying therapy; after cycle 4 five of these 11 patients finally continued with SC-t, 5 with IV-t and 1 progressed. From the 11 patients without any preference 7 received SC-t, 2 receive IV-t and 2 progressed; from the 137 patients preferring SC-t, 125 actually received it, 3 received IV-t and 9 progressed.

Conclusions: Our study shows that 82.5% of patients preferred the SC-t over the IV-t. Treatment choice was not influenced by the accompanying therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-17

Title: BELIS: Safety and tolerability of at home administration of trastuzumab (Herceptin®) subcutaneous for the treatment of patients with HER2-positive early breast cancer

Cocquyt VF F, Martinez-Mena CL L, Martens MT T, D'Hondt RG G, Graas M-PL L, Evron E, Fried G, Ben-Baruch NE E, Dijkstra AC C and Van De Walle EI I. UZ Gent, Belgium; CHU St Pierre, Belgium; AZ Turnhout, Belgium; AZ Damiaan, Belgium; Clinique Saint-Joseph, Belgium; Assaf Harofeh, Israel; Rambam Medical Center, Israel; Kaplan Medical Center, Israel and Roche.

Body: Background: Trastuzumab (Herceptin®; H) is a standard component of adjuvant treatment in patients with HER2+ early breast cancer (eBC) and is supported by all major treatment guidelines. The efficacy and safety of Herceptin intravenous (H IV) and Herceptin subcutaneous (H SC) have been shown to be comparable. The main advantage of SC administration is its shorter administration time. Administration at home for selected patients will allow greater independence and may lead to an improved quality of life. In this study, we assessed the safety and tolerability of H SC administered at home by a healthcare professional (HCP) in patients with HER2+ eBC.

Methods: Patients with HER2+ eBC who previously completed 6 cycles of H IV could be included in the study to receive 12 additional cycles of H for a total of 18 cycles. The 12 additional 3-week cycles consisted of 3 cycles of H IV in the hospital (6 mg/kg; cycles 7 to 9; period 1); 3 cycles of H SC in the hospital (600 mg; cycles 10 to 12; period 2); and 6 cycles of H SC administered in the home by a HCP (600 mg; cycles 13 to 18; period 3). Patients are being followed for a total of 24 months after their last treatment. Safety is being assessed from the adverse events (AEs) reported during the study. Patient-reported outcomes were obtained from validated questionnaires for: the satisfaction and quality of the treatments and care, and for symptom severity (0 [absent] to 10 [worst]). HCPs also reported on their experiences with both treatments.

Results: A total of 102 patients were treated in the study between November 2013 and July 2015 and will be followed for safety and efficacy through July 2017. The primary analysis reported here was done after the last 4-week post-treatment follow-up was completed (September 2015). Patient mean age at baseline was 54.4 ± 12.3 (SD) years. A total of 91 (89%) patients reported 549 AEs: 535 (97%) of these were grade 1 or 2 and 194 (35%) were considered treatment related. The proportions of patients with at least one related AE were 7% for H IV period 1, 32% for H SC period 2, and 47% for H SC period 3, which was twice as long as periods 1 or 2. A total of 8 serious AEs were reported in 8 patients (2 each in periods 1 and 2; 4 in period 3). Prior to the first at-home SC administration (cycle 13), 99% of patients were satisfied to a large or very large extent with the IV and SC treatments at the hospital. At cycle 17, 100% of patients were satisfied to a large or very large extent with the SC treatment at home, and 100% of patients thought treatment at home was beneficial to a large or very large extent. In all 3 treatment periods, maximum mean scores were 3.0–3.8 for the most severe symptoms (fatigue, disturbed sleep, and numbness or tingling). All HCPs considered both administration routes to be fairly easy or very easy, and SC administration to be quicker and require fewer preparation resources.

Conclusions: The safety analyses and patient-reported outcomes recorded in this study indicate that H SC administered by a HCP at home instead of at the hospital was not associated with any new safety signals and was considered beneficial by the patients and HCPs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-18

Title: Pre-clinical antitumor activity, tumor localization, and pharmacokinetics of MP0274, an apoptosis inducing, biparatopic HER2-targeting DARPIn®

Fiedler U, Metz C, Zitt C, Bessey R, Béhé M, Blanc A, Schibli R, Dolado I, Herbst J, Dawson KM M and Kiemle-Kallee J. Molecular Partners AG, Schlieren, Zurich, Switzerland; Paul Scherrer Institut, Center for Radiopharmaceutical Sciences, Villigen, Switzerland; ETH Zurich, Inst. of Pharmaceutical Sciences, Switzerland and F. Hoffmann-La Roche, Basel, Switzerland.

Body: Background: HER2 positivity is an important predictive factor for treatment with anti-HER2 agents in several cancers. However, currently available monoclonal antibody and tyrosine kinase inhibitor drugs rarely achieve full disease control. We have developed a new HER2-targeting molecule with a unique pro-apoptotic mode of action that may provide additional benefit to patients. The DARPIn® MP0274 binds to two distinct non-overlapping HER2 epitopes and to human serum albumin for half-life extension. As previously shown**, *in vitro*, MP0274 induces apoptosis and inhibits proliferation of cells expressing HER2 (IHC3+, IHC2+ and IHC1+) and potently inhibits HER2/HER3 downstream signaling. To support clinical development of MP0274, we tested the potency of MP0274 in several HER2 expressing patient-derived xenograft (PDX) models and investigated tumor localization. In addition, pharmacokinetics (PK) analysis was performed in cynomolgus monkeys.

Methods: Antitumor activity of MP0274 was tested in breast and gastric HER2 expressing PDX mouse models and was compared to standard of care therapies. Tumor localization of MP0274 was studied using an Indium-111 labeled version of MP0274 in a human ovarian adenocarcinoma (SKOV-3) xenograft model by whole-body SPECT/CT imaging. The PK of MP0274 was studied in cynomolgus monkeys (MP0274 is cross-reactive with cynomolgus HER2).

Results: In breast and gastric cancer PDX models, MP0274 showed superior efficacy compared to trastuzumab and lapatinib and equivalent efficacy compared to trastuzumab plus pertuzumab as measured by relative tumor volume. The imaging study with SPECT/CT demonstrated that MP0274 localizes effectively to the HER2-expressing human tumor within 24 h. The PK study in cynomolgus monkeys showed a half-life of ≥ 5 days at doses of 5 and 10 mg/kg while at the lowest dose tested (1 mg/kg) MP0274 had a terminal half-life of 0.4 days. The PK results are indicative of target-mediated clearance that becomes saturated at doses above 1 mg/kg.

Conclusions: MP0274, with its unique pro-apoptotic mode of action, demonstrates excellent activity in preclinical PDX models, fast localization to tumor and a long half-life in cynomolgus monkeys. MP0274 was well tolerated in all studies. These results suggest that MP0274 has the potential to provide additional clinical benefit to patients with HER2-expressing tumors. A GLP repeated dose toxicology study is ongoing and a phase I clinical trial is in preparation.

* DARPins are small repeat proteins, designed to bind targets with high affinity and specificity, which can be combined in a modular fashion to produce multi-functional agents.

** U. Fiedler et al. SABC 2013. Abstract# 1094 & Poster# P4-12-30.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-19

Title: Systematic review of clinical trials for monoclonal antibody biosimilars in HER2-positive breast cancer

Jacobs I, Isakov L, Vana AM M, Coiro J, Zacharchuk C and Ewesuedo R. Pfizer Inc., New York, NY; Pfizer Inc., Cambridge, MA and Pfizer Inc., San Diego, CA.

Body: Background: A number of proposed trastuzumab (TRAS) biosimilars are now in development for HER2-overexpressing breast cancer treatment. The purpose of this study was to systematically collate all published clinical data to assess the weight of available evidence for TRAS biosimilars in HER2-positive (+) breast cancer (BC), and to identify additional studies, not captured in the scientific literature, to support informed decision-making by healthcare stakeholders, as well as to raise awareness of ongoing developments within the field. **Methods:** MEDLINE®, Embase® and ISI Web of Science® were searched from inception to Sept 3, 2015. Conference proceedings (n=17) were searched from 2012 to Jul 31, 2015. Studies disclosing potential use of proposed biosimilars in the treatment of HER2+ BC were screened and categorized by originator and study type (English language only). To assess data strength and validity, risk of bias assessments were conducted. The ClinicalTrials.gov (CT.gov) registry was searched to identify any planned/ongoing/complete biosimilar trials in HER2+ BC. **Results:** On the analysis cut-off date, a total of 7 clinical studies (12 publications) were identified for proposed biosimilars of TRAS. The biosimilars identified for TRAS with published clinical data were BCD-022, CT-P6, FTMB and PF-05280014. For BCD-022, a pharmacokinetics (PK)/safety study in HER2+ BC (N=46) evaluating BCD-022 compared to TRAS, demonstrated equivalence between the two treatments. A PK/safety study (N=174) and comparative safety/efficacy study (N=475) of CT-P6 in HER2+ BC patients both provided evidence of PK equivalence, safety, and efficacy of the proposed biosimilar in this patient population. Bioequivalence of FTMB (versus TRAS) was also reported based on the results of a PK/safety study (N=118) in healthy subjects. For PF-05280014, a PK/safety study (N=105) in healthy volunteers demonstrated equivalence with TRAS. One published comparative safety/efficacy study protocol for PF-05280014 versus TRAS was identified for first-line treatment of patients with HER2+ BC (estimated [E]:N=690), and a second PK/efficacy/safety protocol for neoadjuvant BC treatment (E:N=220) was also published. A number of other planned studies were identified in CT.gov. These included 4 comparative safety/efficacy studies in HER2+ BC evaluating ABP 980 (E:N=808), CT-P6 (N=383/N=532), SB3 (E:N=806) and 2 PK/safety studies (BCD-022 [E:N=206], CT-P6 [E:N=174]) in women with BC. Two studies investigating PK and safety in healthy volunteers were also identified (PF-05280014 [E:N=162], SB3 [E:N=109]). **Conclusions:** This systematic review provides an unbiased synthesis of available evidence for proposed TRAS biosimilars in HER2+ BC, including data to support clinical similarity. The available clinical data for the 4 proposed biosimilars (BCD-022, CT-P6, FTMB and PF-05280014) investigated in a total of 918 healthy subjects or patients indicated highly comparable PK, safety, or efficacy profiles, versus TRAS. Additional data are required to fully evaluate the clinical similarity for proposed TRAS biosimilars, and the completion of several ongoing comparative trials are expected to provide further assurance of safety and efficacy in specific patient populations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-20

Title: Lapatinib after pertuzumab and ado-trastuzumab emtansine in metastatic HER2-positive breast cancer

Báez-Vallecillo L, Singareeka Raghavendra A, Hess K, Moulder SL L, Tripathy D, Valero V and Murthy RK K. The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Lapatinib (L) is approved in combination with capecitabine for patients with trastuzumab-resistant HER2-positive metastatic breast cancer (MBC) and in combination with letrozole for HER2-positive and ER/PR-receptor-positive MBC for whom hormonal therapy is indicated. However, there are no data on the activity of L in patients who received prior pertuzumab (P) and ado-trastuzumab emtansine (TDM-1), now included as standard first and second line therapies. The goal of this study was to assess the efficacy of L in routine clinical practice in a contemporary patient population that received prior P and/or TDM-1.

Methods: We identified all patients with trastuzumab-resistant HER2-positive MBC who received L-based therapy (n=570) between 2003 and 2016 through a departmental database at MD Anderson Cancer Center and reviewed the medical records of each patient to confirm treatment sequencing and outcome. Of these patients, we identified a target cohort who received L after prior P or TDM-1 (n=34). Analysis used Kaplan-Meier statistics.

Results: Of the 34 patients identified as the target cohort with prior P and/or TDM-1 exposure, 29 patients were available for outcome analysis since 5 patients were started on L-based treatment within 4 months of this analysis. In the comparison cohort (n=536) who had received L-based therapy without prior P and/or TDM-1 exposure, 445 patients were available for outcome analysis. In [Table 1], we display the patient characteristics and results. Clinical benefit rate (CBR), defined as complete or partial response or stable disease \geq 6 months, was 58% (95% CI, 39%, 79%) for the target cohort and 78% (74%, 84%) for the comparison cohort. In the target cohort, nearly 25% (n=8) had received \geq 3 lines of therapy and in the comparison cohort only 10% (n=71) had received \geq 3 lines of therapy. The median duration on L was 4.9 months (range 0.7-19.2) in the target cohort and 5.6 months (range 0.0-82.3) in the comparison cohort. In both cohorts, median TTP and OS were longer in patients with de novo disease compared to patients with disease recurrence.

Conclusions: In the target cohort with prior P and TDM-1, nearly one third of patients received L for \geq 6 months and over half the patients obtained clinical benefit for \geq 6 months with no significant toxicity. This suggests clinically relevant benefit to L in a contemporary population of patients who have received prior P and TDM-1. However, clinical benefit and median duration on L were decreased in the target cohort versus the comparison cohort, which may reflect heavier pretreatment.

Table 1: Results

Characteristics	Target Cohort (N=34)	Comparison Cohort (N=536)
Median Age, years (range)	51 (31,78)	52 (24,85)
Median lines of metastatic therapy prior to L, n (range)	2.5(1-6)	1 (0-12)
Discontinuation of L due to toxicity, n (%)	1 (3)	54 (10)
CBR, % (95% CI)	58 (39, 79)	78(74, 84)
Median TTP, months (95% CI)	4.9 (3.0, 7.6)	5.7 (0.0, 82.3)
	De novo	7.4 (2.9, NR)
	Recurrent	4.5 (2.9, 8.3)
Median OS, months (95% CI)	23.9 (20.5, NR)	25.8 (23.1, 30.6)
	De Novo	26.1 (23.9, NR)
	Recurrent	20.5 (12.7, NR)

Abbreviations: NR – not reached; CI – confidence interval

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-21

Title: Her2 is not a cancer subtype but rather a driver found in all intrinsic subtypes and highly enriched in molecular apocrine tumors

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Body: Her2-enriched (Her2E) breast tumors defined by molecular profiling are discrepant from Her2-amplified tumors defined by clinical assessment – over half of all Her2-amplified tumors are not Her2E, while one-third of Her2E tumors are not Her2-amplified. We explored this discordance using genomic profiles of 4,000 breast tumors. We find clear support that Her2 amplification is a discrete cancer driver event and not a subtype, with little genomic impact other than in expression of genes on the amplicon. Many Her2-amplified tumors clearly belong to the basal, luminal A, or luminal B subtypes. After accounting for the Her2 amplicon, most tumors classified as Her2E appear to be molecular apocrine, expressing high levels of androgen receptor and low to moderate levels of ER. The high rate of Her2 amplification in this group (63%) suggests that additional growth drivers are required for molecular apocrine tumors. Her2 amplification is also a driver across subtypes of several non-breast tumor types. Her2 is amplified in 2% of 5,400 non-breast tumors, spanning 23 tumor types. The Her2 region in non-breast cancers shows a similar range of amplification and breadth of the amplicon, and a comparable rate of overexpression of genes within the amplicon for most Her2A tumors. These discoveries reveal therapeutic opportunities for combining anti-Her2 therapy with endocrine or anti-androgen agents for breast cancer, and for expanding anti-Her2 therapy to other tumor types.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-22

Title: Oncogenic potential of ERBB3 mutations in human mammary epithelial cells

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Body: Activation of the HER (ErbB) family of receptor tyrosine kinase signaling is common in patients with metastatic breast cancer. HER2, a member of ErbB family is over-expressed in 25% of breast cancers. A recent large-scale genomics study reported that HER3 (ErbB3) mutations play a significant role in the development of acquired resistance in breast cancer patients. We are examining the role of HER3 mutations in the context of HER2-driven and ER-driven breast cancers, both *in vitro* and *in vivo*. We introduced a series of HER3 mutations (F94L, G284R, D297Y, D313H, K329T, T355I, L792V, E1261A), identified in breast cancer patients, using site-directed mutagenesis. Mutant HER3 constructs were subcloned into the Gateway-compatible lentiviral expression vector pDONR223-ErbB3 which were subsequently subcloned into the Gateway-compatible lentiviral pLX302 destination vector. Stable cell lines were generated in MCF10A and MCF10A/HER2 and ER+ MCF-7 and T47D breast cancer cells using lentiviral transduction. We confirmed the presence of HER3 constructs by digestion with BsrGI restriction enzyme and v5-tagged protein expression. We evaluated the oncogenic potential of these cell lines using various cell based assays. Out of the above listed mutations, T355I mutation was transforming in the breast cancer cell lines. In parallel experiments, we tested the effect of knocking down HER3 in tumor cell lines that harbor endogenous HER3 mutations. The HER3 K742E mutation occurs endogenously in the IGROV-1 (ovarian) and P262H & V104M in SNU-407 (colorectal), A232V in SNU-1040 (colorectal), N126K and R667H in HCT-15 (colorectal) cancer cell lines. We transfected the cells with either HER3 or control siRNA and determined that each cell line had a reduction in proliferation with knockdown of HER3. These data further suggest the potential for these HER3 mutations to be oncogenic. Furthermore, we investigated the signal transduction pathways in wild-type (wt) and mutant (mut) HER3 in ER+ MCF-7 and T47D as well as MCF10A and MCF10A/HER2 breast cancer cells using clinically relevant inhibitors against ER and HER2. In ongoing experiments, the wt and mut ER+ MCF-7 and T47D stable cells are subjected to ER specific inhibitors, fulvestrant or 4-hydroxytamoxifen. In separate experiments, MCF10A/HER2 cells are being treated with the HER2 inhibitor, lapatinib and HER2-mediated transformation is being assessed by colony formation, cell migration/invasion and three-dimensional growth assays. We are also investigating the signaling pathways responsible for the oncogenic transformation of these cell lines. Accordingly, cell lysates prepared from MCF-7 and T47D or MCF10A/HER2 are being analyzed for Akt, Erk, and ErbB activation. These studies will provide the first systematic assessment of how mutations in HER3 affect response of HER2+ or ER+ breast cancers to clinically relevant inhibitors, using a library of naturally occurring HER3 mutants.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-23

Title: A retrospective evaluation of cardiovascular recovery and oncologic outcomes associated with interrupted and continuous adjuvant trastuzumab in the setting of mild left ventricular dysfunction

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Body: Background: Trastuzumab is a monoclonal antibody that targets the HER2 receptor. In the adjuvant setting, 12 months of trastuzumab therapy has been shown to significantly reduce the risk of cancer recurrence. However, treatment with trastuzumab may also be associated with a decline in left ventricular (LV) function that, in most cases, will recover after trastuzumab therapy is stopped. When severe or symptomatic left ventricular dysfunction (LVD) occurs, trastuzumab is generally held with the intention of restoring cardiac function. It is less clear how to manage trastuzumab to optimize both cancer therapy and cardiovascular care when mild, asymptomatic drops in LV function are observed. The intention of this study was to assess how trastuzumab was managed immediately after the development of mild LVD in a non-trial setting and evaluate these outcomes.

Methods: Patients who received adjuvant trastuzumab therapy for breast cancer between September 2005 and September 2010 in British Columbia were identified. Heart function imaging data for these patients were subsequently reviewed to identify those who experienced either a drop in LV ejection fraction (LVEF) to 40-49% or a greater than 15% drop in LVEF relative to their pre-chemotherapy baseline to a final LVEF \geq 50%. The charts of patients who met these inclusion criteria were then reviewed for demographic information, comorbidities, trastuzumab dosing regime, subsequent cardiac events, and breast cancer outcomes.

Results: A total of 171 patients were included in this study. 121 (70.8%) patients had an immediate hold in their trastuzumab therapy while the remaining 50 (29.2%) continued with their next scheduled dose. The number of patients who experienced a cancer relapse event in the therapy interruption and continuation groups were 21 (17.4%) and 5 (10.0%), respectively ($P = 0.25$). Cardiovascular events in the therapy interruption group included a subsequent drop in LVEF to $<$ 40% in 11 (9.1%) patients, long-term congestive heart failure (CHF) in 2 (1.7%) patients, and 1 (0.9%) death related to CHF. Cardiovascular events in the therapy continuation group included a subsequent drop in LVEF to $<$ 40% in 3 (6.0%) patients and long-term CHF in 1 (2.0%) patient. 24 (19.8%) patients in the therapy interruption group had a final LV function measurement that had fallen more than 10% below their baseline value while 12 (24.0%) patients in the therapy continuation group met this criterion ($P=0.54$). The mean numbers of adjuvant trastuzumab doses administered in the therapy interruption and continuation groups were 11.3 and 14.3, respectively. Finally, mean follow up times for the therapy interruption and continuation groups were 5.6 and 5.8 years, respectively.

Conclusions: Continuing trastuzumab immediately after the development of mild LVD did not appear to be associated with an increased risk of long-term cardiovascular events. However, holding trastuzumab immediately after the development of mild LVD appeared to be associated with a 7.4% absolute risk increase in breast cancer recurrence. While these results are not statistically significant, they are concerning and warrant further investigation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-24

Title: Development of mathematical prediction models to identify disease-free survival events for HER2-positive primary breast cancer patients treated by neoadjuvant chemotherapy and trastuzumab

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Body: Background)

The addition of trastuzumab to standard neoadjuvant chemotherapy (NAC) doubles the pathological complete response (pCR) rate in patients with HER2-positive primary breast cancer. Patients who achieved pCR after NAC with trastuzumab showed a better prognosis compared to those without pCR. However, it is still difficult to predict the likelihood of recurrence after surgery at an individual patient-level. The aim of this study was to develop a mathematical model to predict disease-free survival (DFS) events such as recurrence for patients treated with NAC and trastuzumab. Because brain metastasis (BM) often occurs in HER2-positive cancer patients and it is a particular event for those, we planned to develop a specific model for BM as well.

Patients and Methods)

Data of 776 HER2-positive primary breast cancer patients from the multicenter cohort study (JBCRG-C03) were used in the analysis. All patients had received NAC plus trastuzumab between 2001 and 2010. Two prediction models using a machine learning method (alternating decision tree algorithm) were developed using age, body-mass index, menopausal status, clinical stage, histological type, ER/PgR status, histological/nuclear grade, type of surgery, pathological response, adjuvant radiation therapy, and adjuvant hormonal therapy. The model A (DFS) predicted the probability of any disease recurrence, death by any cause, or secondary malignancy within 5 years after starting treatment. The model B (BM) predicted the probability of occurrence of BM within the 5 years. First, bias-controlled virtual datasets were generated for the training of the models using a resampling method. Second, the models were optimized by cross-validation (CV). Finally, the developed models were validated using the original dataset. The area under the receiver operating characteristics curve (AUC) was calculated to assess the discrimination ability of the models.

Results)

The DFS and BM event was observed in 118 and 30 patients, respectively. The AUC values for the model A and model B were 0.833 (95% CI, 0.798–0.868, $P < 0.001$) and 0.927 (95% CI, 0.905–0.949, $P < 0.001$), respectively. The sensitivity and specificity at the cut-off value of 50% were 72.0% and 78.4% for the model A, and 100% and 83.7% for the model B, respectively. Patients predicted as “low-risk” by the model A showed a significantly better 5-year DFS rate than “high-risk” patients (91.2% vs 53.8%, $P < 0.001$). Patients predicted as “low-risk” by the model B showed a significantly better 5-year BM-free survival rate than “high-risk” patients (100% vs 76.1%, $P < 0.001$). The discrimination ability of these models were maintained for both ER/PgR-positive and ER/PgR-negative subgroups, and also for both pCR and non-pCR subgroups.

Conclusions)

Our models showed high accuracy for predicting DFS events and BM in HER2-positive primary breast cancer patients treated with NAC and trastuzumab. These two models would help to realize accurate prediction of DFS events and to optimize the postoperative surveillance plan. The identification of high-risk patients for recurrence including BM may be useful for selecting a patient-subpopulation who requires new therapeutic approach.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-25

Title: The importance of hormone receptor status on biomarker expression and the efficacy of lapatinib plus capecitabine therapy after progression on trastuzumab in HER2 positive recurrent and advanced breast cancer

Arima N, Nishimura R, Toh U, Tanaka M, Saimura M, Okumura Y, Saito T, Tanaka T, Teraoka M, Shimada K, Koga T, Kurashita K, Todoroki H, Ueo H, Ohi Y, Toyoshima S, Mitsuyama S and Tamura K. Kumamoto Shinto General Hospital, Kumamoto, Japan; Kumamoto Shinto General Hospital, Kumamoto, Japan; Kurume University School of Medicine, Kurume, Fukuoka, Japan; JCHO Kurume General Hospital, Kurume, Fukuoka, Japan; Kitakyushu Municipal Medical Center, Kitakyushu, Fukuoka, Japan; Kumamoto City Hospital, Kumamoto, Japan; Saitama Red Cross Hospital, Saitama, Japan; Fukuoka University, Fukuoka, Japan; Sagara Hospital, Kagoshima, Japan; Shimada Breast Clinic, Kitakyushu, Fukuoka, Japan; Hirose Hospital, Fukuoka, Japan; Urasoe General Hospital, Urasoe, Okinawa, Japan; National Hospital Organization Kokura Medical Center, Kitakyushu, Fukuoka, Japan; Ueo Breast Cancer Hospital, Ooita, Japan; Sagara Hospital, Kagoshima, Japan; Kitakyushu Municipal Medical Center, Kitakyushu, Fukuoka, Japan and General Medical Research Center School of Medicine, Fukuoka University, Fukuoka, Japan.

Body: Background: Anti-HER2 treatment using trastuzumab (Tmab) has contributed to improving the clinical outcome of HER2-positive breast cancer patients. However, some patients do not respond to Tmab therapy and the combination of Lapatinib and capecitabine (LC) is an effective treatment option after progression on Tmab. Hormone receptor status is also an important factor for deciding if the patient should be treated with endocrine therapy as well. The aim of this study was to investigate the clinical significance of hormone receptor status in biomarker expression and to evaluate the efficacy of lapatinib therapy. Materials and Methods: Eighty patients with HER2 positive breast cancer refractory to Tmab were enrolled in this prospective trial (KBC-SG 1107) between December 2011 and March 2014. The following treatment began after enrollment; lapatinib 1250-mg tablets were administered orally once daily and capecitabine (2000 mg/m² per day) on days 1 to 14 every 21 days until disease progression or until severe adverse events. Total HER2 (H2T), p95HER2 (p95), and total HER3 (H3T) expression levels were quantified in formalin-fixed paraffin embedded samples using VeraTag assays. ER and progesterone receptor (PgR), PTEN and p95 expressions were evaluated using immunohistochemistry (IHC) and PIK3CA mutation using direct sequencing. Statistical analyses were performed using SPSS (ver. 21). A two-sided P<0.05 was considered a statistically significant difference. Results: The ER- and PgR-positive rates were 55.0% and 33.8%, respectively. The response rate to LC was 30% (CR: 1 case; PR: 23 cases), the clinical benefit rate was 51.3% and the median progression-free survival (PFS) was 174.5 days. Both ER and PgR negativity significantly correlated with higher H2T (cutoff: 13.8), p95HER2 (cutoff: 2.8) and PTEN expression levels (cutoff: H score of 100). Lower H2T expression levels and PIK3CA mutation rates were often observed in the non-responders (both: p=0.087). The ER and PgR status did not correlate with response. A high p95 and PTEN expression significantly correlated with longer PFS in ER and/or PgR positive cases (p=0.02 and 0.03), respectively. The overall survival (OS) after LC significantly correlated with the number of recurrence organs (p=0.0002) but not with the p95 and PTEN expression levels. Conclusion: LC therapy was effective in Tmab-refractory HER2 positive breast cancer. Moreover, the biomarker expression differed depending on the ER/PgR status and a high p95 and PTEN expression correlated with longer PFS in ER and/or PgR positive cases. Further study is necessary to validate these findings.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-26

Title: Population pharmacokinetics (PK) and exposure-response (E-R) analysis of trastuzumab emtansine (T-DM1) in patients with HER2+ metastatic breast cancer (MBC) who have received at least two prior regimens of HER2-directed therapy

Chen S-C, Polhamus D, Riggs M, French J, Wang X, Smitt M, Hoersch S, Strasak A, Chernyukhin N, Quartino A, Jin J, Girish S and Li C. Genentech, Inc., South San Francisco, CA; Metrum Research Group, Tariffville, CT and Roche, Basel, Switzerland.

Body: Background:

TH3RESA was a Phase III randomized study to evaluate the efficacy and safety of trastuzumab emtansine (T-DM1) compared to treatment of physician's choice (TPC) in patients with HER2+ MBC who have progression after at least two regimens of HER2-directed therapy. Population pharmacokinetic (PK) and exposure-response (E-R) analyses were performed to characterize T-DM1 PK as well as E-R relationship for key efficacy and safety endpoints in the population.

Methods:

Post-hoc analysis based on historical T-DM1 population PK models was performed to assess whether PK is consistent with historical data. E-R analyses with OS and PFS were conducted using Cox proportional hazard (CPH) models with exposure metrics (model-predicted Cycle 1 C_{min} and AUC_{ss}) included in the model. Logistic regression models were used for binary endpoints of overall response rate (ORR) and key safety endpoints with exposure metrics included as continuous variable only. To supplement the E-R analysis for OS and PFS, case matching analyses were conducted to compare OS and PFS in the lowest exposure quartile (Q1) vs. higher exposure quartiles (Q2-4) to their corresponding matched control.

Results:

Historical T-DM1 population PK model well described T-DM1 PK in TH3RESA study. In CPH analyses with OS and PFS, hazard ratios (HR) of both efficacy endpoints consistently decreased with increasing T-DM1 exposure

HR for Quartile of C_{min} Before and After Adjusting for Risk Factors

	Quartile	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
OS	Q1	1.14 (0.832, 1.55)	0.886 (0.64, 1.23)
	Q2	0.828 (0.6, 1.14)	0.685 (0.493, 0.952)
	Q3	0.532 (0.374, 0.757)	0.559 (0.391, 0.798)
	Q4	0.352 (0.238, 0.521)	0.405 (0.272, 0.603)
PFS	Q1	0.852 (0.63, 1.15)	0.831 (0.614, 1.12)
	Q2	0.635 (0.463, 0.872)	0.619 (0.451, 0.85)
	Q3	0.428 (0.3, 0.609)	0.442 (0.31, 0.632)
	Q4	0.237 (0.158, 0.357)	0.258 (0.171, 0.389)

and

HR for Quartile of AUC_{ss} Before and After Adjusting for Risk Factors

	Quartile	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
OS	Q1	1.07 (0.782, 1.47)	0.774 (0.555, 1.08)
	Q2	0.651 (0.464, 0.912)	0.567 (0.402, 0.801)
	Q3	0.662 (0.472, 0.929)	0.736 (0.523, 1.04)
	Q4	0.406 (0.280, 0.588)	0.458 (0.315, 0.667)
PFS	Q1	0.69 (0.505, 0.941)	0.657 (0.481, 0.897)

	Q2	0.66 (0.479, 0.908)	0.681 (0.494, 0.940)
	Q3	0.524 (0.377, 0.730)	0.545 (0.390, 0.760)
	Q4	0.235 (0.155, 0.355)	0.253 (0.167, 0.383)

. The E-R relationship is supported by case matching analyses, where T-DM1 treated patients were stratified by model-predicted Cycle 1 C_{min} . HRs of OS and PFS for patients at Q2–4 versus their matched TPC patients (HR (95%CI): 0.58 (0.44, 0.78) for OS; 0.47 (0.36, 0.62) for PFS) were numerically smaller than that of T-DM1 treated patients at Q1 versus their corresponding matched TPC patients (HR (95%CI): 0.96 (0.63, 1.47) for OS; 0.92 (0.64, 1.32) for PFS). For ORR, an E-R trend was also noted. On the other hand, no E-R relationship was identified with key safety endpoints.

Conclusion:

T-DM1 PK in TH3RESA patient population is similar to historical data. Although an E-R relationship was observed for efficacy, the results need to be interpreted with caution given the potential confounding association between risk factor and PK. No E-R relationship was observed for the safety endpoints examined.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-27

Title: Plasma chemokine profile of HER2+ breast cancer (BC) patients treated with docetaxel (D) and carboplatin (C) in combination with trastuzumab (T) and/or lapatinib (L) in the neo-adjuvant setting

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Body: Background: Chemokines (chemotactic cytokines) are key regulators of the immune response, attracting immune cells to sites of inflammation, activating adhesion molecules, promoting extravasation and influencing T-cell subset polarization. Recent clinical data has shown a strong correlation between tumor infiltrating lymphocytes (TILs) and response to T therapy in HER2+ BC while the combination of chemotherapy, T and L has proven superior to chemotherapy and T alone in the neo-adjuvant setting (NeoALTTO). This preliminary study examines the levels of 40 circulating chemokines in 32 HER2+ BC patient plasma samples from ICORG 10-05 (neo-adjuvant DCT, DCL or DCTL) to investigate differences in chemokine profiles between treatment arms and patient's response to treatment.

Methods: Pre-treatment blood samples were collected before commencement of chemotherapy; post-treatment samples were collected two weeks prior to surgery. All patients received G-CSF concurrent with treatment. A panel of 40 chemokines and chemokine-associated cytokines were assessed by Luminex xMAP multiplex assay. Matched patient samples were run in single replicates and analysed together (n=32) or by comparing DCT (n=12) and DCTL (n=14) treatment arms (using a paired Mann-Whitney test and adjusting for multiple testing using Bonferroni correction). DCL (n=6) was omitted from the arm comparison due to low numbers. In addition, principle components analysis (PCA) was used to explore any trends within the data based on patient response (pathological complete response (pCR, n=15), partial response (PR, n=12), and non-response (NR, n=4)). 12 non-age matched healthy volunteer controls were also included in the PCA.

Results: PCA shows a clear separation between pre- and post-treatment samples. 30 of the 40 chemokines examined were significantly differentially expressed (adjusted p-value of <0.05) post-treatment across all treatment arms. When comparing DCT and DCTL, nine chemokines were significantly altered post-therapy in both arms with two chemokines, CCL24 (p=0.039) and IL-16 (p=0.039), increased in DCT only and ten chemokines, CCL11 (p=0.009), CX3CL1 (p=0.004), CXCL1 (p=0.009), CCL1 (p=0.024), IL-2 (p=0.043), IL-6 (p=0.034), IL-8 (p=0.004), CXCL11 (p=0.004), CXCL16 (p=0.004) and TNF-alpha (p=0.004) altered in DCTL only. PCA identified no trend between pre- and post-treatment chemokine levels and response.

Conclusions: DCT and DCTL produce statistically different alterations in the plasma chemokine profiles of HER2+ BC patients. Pre- or post-treatment levels of the chemokines examined are not collectively predictive of patient response to treatment. Further work is required to elucidate the relevance of DCT- and DCTL-specific chemokine alterations to response.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-28

Title: Trastuzumab improves outcomes of New Zealand women with HER2+ stage I-III breast cancer

Lawrenson R, Lao C, Harvey V, Campbell I, Brown C, Seneviratne S, Edwards M, Scott N, Elwood M, Sarfati D and Kuper-Hommel M. The University of Waikato; The University of Waikato; Auckland Breast Cancer Registry; Waikato Breast Cancer Registry; The University of Waikato; University of Colombo; Waikato District Health Board; Waikato District Health Board; University of Auckland; University of Otago and Waikato District Health Board.

Body: Introduction

HER2 status has been routinely ascertained for stage I-III breast cancer since late 2005. Trastuzumab was first funded in New Zealand for use in HER2+ breast cancer in July 2007. This observational study aims to compare the difference in outcome between women with HER2+ stage I-III breast cancer who received trastuzumab as adjuvant therapy versus those who did not. Differences in presentation, treatment and outcomes between Māori and NZ European were studied.

Methods
The combined Waikato and Auckland Breast Cancer Registries have clinical details of 12377 women diagnosed with breast cancer between June 2000 and May 2013. 9506 women with breast cancer were tested for HER2 receptor status. Proportion of women with HER2+ (FISH amplified or IHC 3+), stage I-III breast cancer were examined by age, ethnicity and stage. Differences in use of trastuzumab for women with stage I-III breast cancer with a tumour size equal or larger than 1 cm and aged less than 75 years were assessed by ethnicity and year of diagnosis. Patients who had inflammatory breast cancer or developed metastatic disease or local recurrence within 3 months after diagnosis were excluded. Kaplan-Meier method and Cox proportional hazards model were used to examine the breast cancer-specific survival between women treated with trastuzumab and chemotherapy and those treated with chemotherapy without trastuzumab.

Results
1454 patients with early invasive breast cancer were HER2+. The proportion of cases with HER2+, stage I-III breast cancer increased with stage (stage I-III: 11.5%-26.9%), but decreased with age (<40 years to 80+ years: 28.8%-9.7%). Māori women were more likely to have HER2+ cancers than NZ European (17.8% versus 14.9%; $p=0.02$). Among the eligible patients, 605 women received trastuzumab and chemotherapy within 12 months for stage I-III breast cancer, and 275 had chemotherapy without trastuzumab. A small proportion (10.2%, 34/333) of women diagnosed in 2000-2005 received trastuzumab as part of a clinical trial. The proportion of women who received trastuzumab increased to 60.3% in 2006-2009 and to 87.1% in 2010-2013. 46.2% of Māori women were treated with trastuzumab compared to 55.9% of NZ European ($p=0.040$). The cancer-specific survival estimated with Kaplan-Meier method is shown in Table 1. Women treated with chemotherapy without trastuzumab were 2.7 times (95% CI: 1.9-3.9) more likely to die of breast cancer compared to those treated with trastuzumab and chemotherapy, after adjustment for stage, tumour size and hormone therapy.

Table 1. Breast cancer-specific survival between women with HER2+ stage I-III breast cancer who received trastuzumab as adjuvant therapy versus those who did not

Treatment	Number of patients	Median follow-up time (months)	5-year survival	10-year survival
Trastuzumab+chemotherapy	605	53	89.6%	84.3%
Chemotherapy without trastuzumab	275	107	75.6%	69.1%

Conclusions

Trastuzumab improved the breast cancer-specific survival of women with HER2+ stage I-III breast cancer. Māori women were more likely to have HER2+ cancer and less likely to be treated with trastuzumab. Rates of treatment with trastuzumab and the adjusted survival between Māori women and NZ European women were not significantly different.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-29

Title: HER2-associated lipogenic phenotype as a potential therapeutical target in breast cancer patients

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Body: Lipogenic phenotype (genetic program activation involved *de novo* fatty acid –FA-synthesis and key metabolic regulators) is associated to oncogenic transformation and malignancy. We hypothesize that in HER2 overexpressing breast cancer cells, HER2 function is affected by cell membrane lipid profile, mainly lipid rafts, necessary to HER2 location and activation within the cell membrane. Therefore, metabolic lipid imbalance could be a potential factor and marker associated to HER2 expression. To evaluate if cell lipogenesis and membrane lipid composition are affected by HER2 overexpression, we used an oncogenic transformed cell line engineered to overexpress HER2 receptor (HB4aC5.2), but identical to their parental strain (HB4a) in all other aspects. Several lipid related molecules including synthesis-FASN, uptake-*CD36*, transport-*FABP4* and FA storage-*DGAT* by RT-PCR; and lipogenic regulatory pathways (mTOR, DEPTOR, SREBP1 and PPAR γ) were evaluated in both cells. Lipogenic contribution to lipid rafts formation was evaluated by gas chromatography and confocal microscopy. The influence of HER2 overexpression and lipogenic phenotype on proteins activated by HER2 (AKT, ERK1/2 and FASN) was analyzed by western blot. Next, HB4a and HB4aC5.2 cells were treated, alone or in combination with DHA (omega-3 polyunsaturated fatty acid), by Trastuzumab (anti-HER2), and GW9662 (anti-PPAR γ) for 72h, and all above experiments were repeated. Cell death was analyzed by flow cytometry. *In vitro* results were confirmed in Primary tumor and adjacent tissue samples from a cohort of 182 European breast cancer patients through metabolomics analysis. Statistical analysis was performed by MetaboAnalyst software ($p < 0.05$). In HB4aC5.2 cells, the HER2 overexpression was associated with a lipogenic phenotype, which increase in cholesterol and saturated FA synthesis for the lipid rafts formation, suggesting that large amounts of HER2 require cell membrane changes to favor rafts saturation and formation, when compared to HB4a. Lipid rafts were associated with survival and proliferation signals hiper activation ($p < 0.001$). Cell lipogenesis was independent of mTOR/SREBP/PPAR γ pathway and occur concomitantly to DEPTOR (mTOR inhibitor) increased expression. Moreover, GW9662 or Trastuzumab treatment did not influence lipogenesis in cell culture. In HB4aC5.2 cells, only DHA treatment decreased the lipogenic phenotype, DEPTOR expression, saturated FA synthesis, disrupted lipid raft, inhibited HER2 signaling, and induced apoptosis ($p < 0.001$). In addition, when added to Trastuzumab DHA potentiated cell death. In tumor tissue samples an increased FA synthesis was observed, such as palmitic acid, sphingolipids and phosphatidylcholines saturated FA enriched, synthesis of cholesterol, suggesting an increase of lipid rafts when compared to normal breast adjacent tissue. Our results indicate that lipogenic phenotype is associated with HER2 superexpression in breast cancer cells, and it seems important to promote survival and proliferation, through lipid rafts increase. DHA modulation increased death in tumor cells, suggesting that cell membrane lipogenesis and lipid composition may represent a treatment target. Lipogenic phenotype confirmation in tumor tissue samples strengthens this hypothesis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-30

Title: Long-term survival in HER2-positive metastatic breast cancer: The first blow is half the battle

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Body: Introduction: Metastatic breast cancer (MBC) is considered an incurable disease. However, long-term survival is increasingly observed in HER2-positive disease since the introduction of trastuzumab. We explored factors associated with long-term survival in a retrospective series at our Institute.

Methods: All patients with histologically proven HER2-positive (3+ score by IHC or HER2 amplification) MBC treated starting first-line trastuzumab-based palliative therapy between January 2003 to January 2013 were included. Patients were identified from the Institute's tumor registry and data were collected from patient records. The primary endpoint was overall survival. Kaplan-Meier survival estimates were calculated and multivariable survival analyses were performed to identify independent prognostic factors. Radiologic complete response (CR) was a secondary endpoint.

Results: We identified 113 patients with a median age at diagnosis of MBC of 52 years (range 27-82). Median follow-up for MBC was 39 months (range 2-148 months). Thirty-eight percent presented with synchronous metastases; 62% had recurrent disease of whom 42% had received prior trastuzumab as part of (neo-)adjuvant treatment. First-line palliative treatment consisted of trastuzumab plus vinorelbine (56%), a taxane (28%), capecitabine (10%), other chemotherapy (3%), or endocrine therapy (2%), and resulted in a CR in 27 patients (24%). In addition, one patient achieved CR to third-line therapy. Most patients with CR had received trastuzumab with a taxane (57%) followed by vinorelbine (32%). Fourteen out of 28 patients with CR are still alive without evidence of disease at a median follow-up of 91 months (range 23-148 months), of whom 8 still receive trastuzumab. Fourteen patients had disease relapse (8 on maintenance trastuzumab, 6 after discontinuation). Overall, 35 patients (31%) survived more than 5 years. Factors associated with long-term survival in univariable analyses were oligo-metastatic disease (1-3 distant metastases), synchronous metastases, no skin or brain metastases, no prior (neo-)adjuvant trastuzumab, first-line palliative treatment with trastuzumab and taxanes, and achieving a radiologic CR on treatment (see table 1). Achieving CR and the absence of skin metastases remained significant factors in multivariable analyses.

Conclusion: Thirty-one percent of patients with metastatic HER2-positive MBC survive over 5 years. Long-term response is particularly seen in patients who achieve a complete radiologic response on first-line treatment. This finding supports a strategy to administer the most effective agents as first line treatment, as is often but not always applied in clinical practice.

Table 1. Prognostic factors associated with overall survival

	Univariable			Multivariable		
	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Synchronous metastases						
No	-			-		
Yes	0.44	(0.27-0.70)	<0.001	0.80	(0.46-1.40)	0.43
Oligo-metastatic disease						
No	-			-		
Yes	0.37	(0.22-0.62)	<0.01	0.66	(0.36-1.17)	0.14
Skin metastases						
No	-			-		
Yes	5.40	(2.91-10.01)	<0.01	4.10	(2.08-8.14)	<0.01
Brain metastases						
No	-			-		

Yes	2.25	(1.03-4.93)	<i>0.04</i>	1.46	(0.55-3.98)	<i>0.45</i>
(Neo-)adjuvant trastuzumab						
No	-			-		
Yes	1.87	(1.16-2.99)	<i>0.01</i>	1.66	(0.93-2.96)	<i>0.09</i>
Trastuzumab + taxane						
No	-			-		
Yes	0.54	(0.32-0.89)	<i>0.02</i>	0.69	(0.40-1.20)	<i>0.19</i>
Complete radiologic response						
No	-			-		
Yes	0.13	(0.06-0.29)	<i><0.01</i>	0.22	(0.09-0.53)	<i>0.001</i>

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-31

Title: Updated safety results from the first multicenter, open-label, phase IIIb study investigating the combination of pertuzumab with subcutaneous trastuzumab and a taxane in patients with HER2-positive metastatic breast cancer (SAPPHIRE)

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Body: Background: Pertuzumab targets the human epidermal growth factor receptor 2 (HER2) through an independent epitope to that of trastuzumab. Improved efficacy with acceptable toxicity has been shown, in metastatic breast cancer (mBC) for pertuzumab in combination with intravenous (IV) trastuzumab and docetaxel.¹ This study aimed to assess the safety, tolerability and efficacy of combining IV pertuzumab with subcutaneous (SC) trastuzumab and a taxane, as 1st-line therapy in patients (pts) with HER2+ mBC. We present safety data with 19 months (mo) of median follow-up.

Methods: SAPPHIRE (NCT02019277) is a multicentre, open-label, phase IIIb study. Primary objective: safety and tolerability of IV pertuzumab + SC trastuzumab and investigator's choice of taxane. Pts ≥18 years with confirmed HER2+ mBC and ECOG PS 0-2 were included. The incidence and severity of adverse events (AEs), serious (S) AEs and AEs leading to premature discontinuation of study treatment were analyzed by taxane group.

Results: 50 pts enrolled in the study. Taxanes of choice: nab-paclitaxel (NP; n=36), docetaxel (D; n=13) and paclitaxel (P; n=1). As of March 4th 2016, 34 pts had withdrawn from study; median follow-up= 19mo (range: 6-27). 28(56%) pts received >18 cycles of study medication. All patients experienced AEs with 326 AEs in the D group and 675 in the NP group; the majority being grade 1-2. The most common AEs were diarrhea, fatigue, peripheral neuropathy, alopecia, and rash. Grade 3+ AEs (n=77) were reported in 30 (60%) pts, 20 in the D group (8 pts; 62%) and 57 in the NP group (22 pts, 61%). The most common grade 3+ AEs in the D group were febrile neutropenia (4 pts, 31%) and neutropenia (3 pts; 23%); and in the NP group neutropenia (3 pts; 8%), anemia, diarrhea, cellulitis, peripheral neuropathy, LVEF decreased and pulmonary embolism each reported in 2(6%) pts. SAEs (n=44) were reported in 25(50%) pts, 11 (8 pts; 62%) in the D group and 33 (17 pts; 47%) in the NP group. The most common SAEs in the D group were febrile neutropenia (4 pts, 31%) and pyrexia (2 pts, 15%); and in the NP group: pyrexia (5 pts, 14%), cellulitis (2 pts, 6%) and pulmonary embolism (2 pts, 6%). AEs of suspected cardiac disorders (n=12) were reported in 7(14%) pts; atrial fibrillation, cardiomyopathy, myocardial ischemia, and dyspnea each reported in 1(2%) pt; and LVEF decreased, palpitation, hypertension each reported in 2(4%) pts. AEs leading to study drug discontinuation (n=6) were reported in 6(12%) pts [LVEF decreased (3), drug hypersensitivity, syncope and blister]. AEs leading to chemotherapy discontinuation (n=24) were reported in 10(20%) pts. 4(8%) pts died on study (disease progression).

Conclusion: No new safety signals have been reported in this study. Of further clinical interest is the finding that no cases of febrile neutropenia were reported in the NP taxane group compared to 4 in the D group. The safety profile remains consistent with the CLEOPATRA,¹ PERUSE² and Hannah³ studies, indicating the combination is safe and tolerable.

1. Baselga, et al. NEJM 2012;**366**:109

2. Bachelot, et al. JCO 2014;32:5s(abstr#548)

3. Ismael, et al. Lancet Onc 2012;**13**:869.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-32

Title: Treatment of early HER2-positive breast cancer in trastuzumab era in everyday clinical practice: An overview after 10 years of its approval

Ortega A, Domenech M, Falo C, Gil M, Stradella A, Fernandez A, Morilla I, Villanueva R, Castany R, Vazquez S, Molina K, Bergamino M, Navarro V and Pernas S. Catalan Institute of Oncology, Hospitalet del Llobregat, Catalonia/Barcelona, Spain.

Body: Introduction: The addition of Trastuzumab(T) to chemotherapy (CT) revolutionized HER2-positive breast cancer(BC) and changed its natural history. We reviewed the efficacy of T outside clinical trials in a cancer comprehensive center.

Methods: Ambispective and descriptive study was conducted in Catalan Institute of Oncology (ICO-Barcelona). Estimates of progression-free survival (PFS) and overall survival (OS) were obtained with the Kaplan-Meier method and compared with LogRank test. The association of clinic-pathological variables and outcome was studied by χ^2 and Cox proportional hazard analysis.

Results: 430 consecutive early HER2-positive BC patients (pts) were treated with adjuvant/neoadjuvant T and CT from Jan 2005 to Dec 2012. Pt basal characteristics are reported in Table 1. Neoadjuvant treatment was administrated in 230pts (54%) and in 200 (46%) in adjuvancy. Pathological complete response (pCR) in breast and nodes (ypT0/isypN0) was achieved in 48% of pts, with higher rates in hormone receptor (HR)-negative pts (62 vs 37% p=0.0005). Median duration of T: 10.6 months (m). 28%pts treated with neoadjuvant T+CT who achieved a pCR did not receive adjuvant T. Treatment discontinuation: 38pts (8.8%): 27pts due to cardiac toxicity and 4 relapsed during adjuvant T. In 87%pts, neoadjuvant CT was based on anthracyclines(A) and taxanes. Adjuvant CT: A and taxanes in 57.4%; 14%pts FAC, 15.4% A-CMF and 12% TCH. At a median follow-up of 70m (3-135), 44pts (10.4%) had relapsed: 33pts with distant M1, 9pts with only loco-regional disease and 2pts contralateral HER2-positive BC. M1 location: 46% visceral, 34% bone/lymph nodes and 20% in central nervous system (CNS). PFS was 23.4m(0-88); median OS was not reached; estimated 10 years-OS was 86.5%. Pts treated with A and taxanes had a significantly better OS compared to those treated with other CT (113 vs 98m, p= 0.009). Kaplan-Meier curve showed numerically higher relapses at 10 years in HR-positive pts (83 vs 90% p=0.8). Pts with pCR had significantly better OS (113 vs 104m, p=0.006). Pts with CNS-metastases had a significantly worse OS (13 vs 26m,p=0,02) and those with HR-negative (49 vs 24m, p= 0.033).

Conclusion: In everyday clinical practice, recurrences after adjuvant/neoadjuvant trastuzumab in HER2-positive BC were less than described in the T-pivotal trials, with 10% of recurrences at a median of FU of 70m. In our series, estimated 10 years-OS was 86.5%. Pts treated with A and taxanes had a significantly better OS as well as those pts who achieved a pCR. On the contrary, pts with CNS M1 and those with HR-negative had worse prognosis.

Table 1

Median age	51.9y (27-83)
Stage I / II/ III	106 (25%) / 226 (52%) / 97 (23%)
HR Positive/ Negative	249 (58%) / 181 (42%)

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-33

Title: HER2 activity regulates the pro-trastuzumab immune tumor microenvironment

Tagliabue E, Forte L, Regondi V, Ghirelli C, Aiello P and Triulzi T. Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

Body: It is well known that trastuzumab activity against HER2-positive breast carcinomas (BCs) depends also on immune cells. Our genomic analysis of 53 HER2-positive BCs indicated that those exquisitely sensitive to treatment are addicted to this oncoprotein and are enriched in immune pathways and T-cells infiltration, raising the hypothesis that HER2 regulates immune cells recruitment. The aim of this study was to investigate how HER2 activity contributes to mold tumor microenvironment. Gene expression profile analysis of 53 HER2-pos BCs showed that trastuzumab-sensitive tumors express significantly higher levels of chemokines involved in immune cells recruitment (IFN γ -related CXCLs and CCLs) and higher levels of immune checkpoint ligands (PD-L1, PD-L2) than tumors that do not benefit from trastuzumab. Immunohistochemistry analysis on FFPE tumor specimens showed a significant positive association between tumor cell positivity for CXCL9, CXCL10 and CCL2 and trastuzumab sensitivity. *In vitro* analysis in HER2-positive BC cell lines revealed that while IFN γ -related chemokines (CXCL9 and CXCL10) are not directly regulated by HER2 signaling, CCL2 was significantly up-regulated both at mRNA and protein levels by HER2 stimulation with EGF/HRG and down-modulated by HER2 inhibition with trastuzumab. The EGF-induced increase in CCL2 expression was abrogated by the concomitant treatment with PI3K inhibitor but not with MEK inhibitor, indicating that HER2 regulates CCL2 expression via the PI3K pathway. HER2 signals derived from cell stimulation with EGF/HRG caused also the upmodulation of PD-L1/PD-L2 both at mRNA and protein levels while their inhibition with trastuzumab reduced their levels. Overall, our results support the notion that the activated HER2 oncogene concomitantly induces the expression of CCL2 and PD-L1/PD-L2 regulating infiltration of pro-trastuzumab immune cells and their suppression. Thus, it is likely that activity of trastuzumab in therapy-responsive tumors derives from its ability in relieving suppression of recruited antitumor effector immunity. Supported by Italian Association for Cancer Research (AIRC).

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-34

Title: Phase II study of gemcitabine, trastuzumab, and pertuzumab for HER2-Positive metastatic breast cancer after prior pertuzumab-based therapy

Iyengar NM M, Smyth L, Lake D, Gucaip A, Singh JC C, Traina TA A, DeFusco P, Dickler MN N, Fornier MN N, Goldfarb S, Jhaveri K, Modi S, Troso-Sandoval T, Argolo D, Jack K, Ulaner G, Jochelson M, Baselga J, Norton L, Hudis CA A and Dang CT T. Memorial Sloan Kettering Cancer Center, New York, NY.

Body: Background: The combination of taxanes with trastuzumab (H) and pertuzumab (P) for first line treatment of HER2-positive metastatic breast cancer (MBC) is associated with improved progression-free survival (PFS) and overall survival (OS). Treatment per physician's choice with anti-HER2 therapy after second line therapy is associated with a median PFS of 3 months. While continued use of H in therapeutic combinations after progression on H-based therapy is common, the efficacy of continuing HP-based treatment after progression on P-based therapy is unknown.

Methods: This is a single arm phase II trial of gemcitabine (G) with HP. Eligible patients had HER2-positive (IHC 3+ or FISH \geq 2.0) MBC with prior HP-based treatment and \leq 3 prior chemotherapies. Patients received G (1200 mg/m²) on days 1 and 8 of a q 3 week (w) cycle, and H (8 mg/kg load \rightarrow 6 mg/kg) and P (840 mg load \rightarrow 420 mg) q3w. The primary endpoint is PFS at 3 months. Secondary endpoints include OS, safety and tolerability. An exploratory endpoint is to compare PFS by RECIST criteria versus 18-F FDG-PET response criteria. Using a Simon optimal 2-stage design, 21 patients were enrolled in stage 1. The successful 3-month PFS rate for stage 1 was set at 57% to allow accrual to stage 2 for a total of 45 patients. The study therapy will be considered successful if at least 27/45 (60%) patients are progression free at 3 months.

Results: As of June 9, 2016, 28 patients are enrolled; 21 are evaluable at 3 months and 7 have not had 3-month evaluation. At 3 months, 16/21 (76%) are progression free; 5 patients have progressed. The 3 month-PFS results for evaluable patients will be updated. There are no cardiac or febrile neutropenic events to date. Initially, 5 of 22 (23%) patients required G dose reduction (4 due to grade 3 neutropenia and 1 due to grade 3 vomiting) and the study was amended to lower initial G dose to 1000 mg/m².

Conclusions: The preliminary 3 month-PFS is 76% (95% CI 55% to 89%) in evaluable patients, and updated data will be presented. These findings suggest clinical benefit when P is continued beyond progression.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-35

Title: Phase II trial of pertuzumab, trastuzumab, and nab-paclitaxel in patients (pts) with HER2 overexpressing (HER2+) locally advanced or inflammatory breast cancer (LABC) or untreated stage IV metastatic breast cancer (MBC)

Somlo G, Frankel P, Yeon C, Yuan Y, Yim J, Kruper L, Taylor L, Mortimer J, Waisman J, Jones V, Vito C, Paz B, Huria A, Li D, Gaal C, Tong T and Tummyan L. City of Hope Cancer Center, Duarte, CA.

Body: Background: Pathologic complete response (pCR) to HER2-targeting neoadjuvant therapy (NT) predicts for improved survival (Cortazar et al, Lancet, 2014). The addition of pertuzumab to trastuzumab and docetaxel increased pCR rates, and, as first line treatment for MBC led to longer overall survival ([OS] Swain et al, NEJM 2015). Avoidance of anthracyclines in the adjuvant setting for HER2+ BC reduced the risk of secondary hematologic malignancies without a detriment to OS (Slamon et al, NEJM, 2011). Finally, nab-paclitaxel (nab) might provide an advantage over other taxanes via decreased use of steroids and may lead to increased response rates (RR). We designed a study of pertuzumab (pert), trastuzumab (trast), and nab, testing the feasibility and efficacy of this regimen in the LABC and metastatic breast cancer settings.

Materials and Methods: Pts with Stages II-III LABC received six cycles of NT with pert (day 1 q 21 days), trast, and nab 100 mg/m² (both given IV, weekly). Pts with untreated MBC received the same regimen until progression, toxicities, or patient or physician preference led to stopping therapy. Primary endpoints included pCR (LABC) and RR and progression-free survival (PFS) in MBC. Forty pts with LABC and 25 pts with MBC were to be accrued. The study was designed to test whether the pCR rate of Neosphere (Gianni et al, Lancet Oncol, 2012, > 45.8%) and the PFS rate of CLEOPATRA (median of > 18.5 months) can be matched or exceeded. Procurement of serial samples for assessment of tumor gene expression, circulating tumor cells, miRNA, and serum DNA profiling for exploratory biomarker analysis was carried out.

Results: Twenty-two of 28 already enrolled pts with LABC (clinical stage II:15, stage III: 7) completed NT. The median age was 53 (34-77). The pCR rate was 86% (6/7) for hormone receptor negative (HR-) and 40% (6/15) for HR+ pts, with an overall pCR of 55%. Three pts without pCR following NT had residual BC with a HER2 negative phenotype. Eighteen of 22 pts required nab dose modifications. The most frequent toxicities following NT included elevated liver function tests:27%, peripheral neuropathy:23%, hematological toxicities:17%, diarrhea:18%, infusion reactions:18%. In the MBC cohort there were 13 of 16 enrolled pts with > 2 months of follow-up. The median age was 47 (31-65), 62% had HR+ disease. A CR rate of 4/13 (31%) and confirmed RR of 77% were observed. The median number of cycles with pert, trast, nab was 9 (3+ to 41); 11 of 13 pts required dose modifications or delays (3 of the delays were due to primary breast surgery performed upon response to treatment). At a median follow-up of 19 months, PFS and OS estimates are 63% (95% CI 0.09-0.93), and 89% (95% CI 0.61-1.0).

Conclusion: The non-anthracycline-containing regimen of pertuzumab, trastuzumab, and nab-paclitaxel induced a high pCR rate in HER2+ BC. PFS is encouraging in MBC. Outcome of the fully accrued cohorts inclusive of residual cancer burden scores in the LABC cohort, and correlative data with exploratory biomarker analysis will be presented.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-36

Title: Splenic enlargement and bone marrow hyperplasia in patients receiving trastuzumab-emtansine for metastatic breast cancer

Kosmin M, Makris A, Jawad N, Miles D and Padhani AR R. Mount Vernon Cancer Centre, Northwood, Middlesex, United Kingdom and Paul Strickland Scanner Centre, Northwood, Middlesex, United Kingdom.

Body: Introduction

Trastuzumab emtansine (T-DM1) is an antibody-drug conjugate used for treatment of human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer. An association between T-DM1 and splenic enlargement was noted anecdotally on sequential whole-body MRI (WB-MRI) examinations. A retrospective analysis of WB-MRI examinations of patients on T-DM1 was undertaken to investigate the hypothesis that an increase in splenic volume is due to either a generalised hyperplasia of the bone marrow and reticulo-endothelial system and/or an increase in portal venous pressure.

Methods

12 patients underwent 29 serial WB-MRIs before and during T-DM1 therapy. Splenic volume, portal vein diameter, bone marrow muscle-normalised signal intensity (nSI), water diffusivity (apparent diffusion coefficient, ADC) and fat fraction were measured. Changes in splenic volume were analysed, and correlations between the measured variables were obtained.

Results

An increase in splenic volume was observed in 92% of patients. Mean splenic volume increased from 144cm³ (95%CI 110-177cm³) to 209cm³ (95%CI 161-257cm³) on T-DM1 therapy (p=0.006). Increase in splenic volume correlated with treatment duration (r²=0.71). Increase in normal bone marrow signal was seen (nSI 3.5 to 4.8, p=0.12), along with a decrease in fat fraction (64.3% to 57.3%, p=0.12), and reduced ADC (655µm²/s to 543µm²/s, p=0.11). No consistent changes to portal vein diameter were seen.

Discussion

An increase in splenic volume was consistently observed in patients on T-DM1 therapy. This was unrelated to portal vein changes but correlated with bone marrow hyperplasia. Caution should be applied when assessing metastatic disease in bone to avoid incorrectly attributing T-DM1-related changes in normal bone marrow to disease progression.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-37

Title: Phase I trial of ruxolitinib in combination with trastuzumab in metastatic HER2 positive breast cancer

Mundi PS S, Lee S, Chi D, Bhardwaj A, Makower D, Cigler T, Crew KD D, Hershman DL L, Califano A, Silva J and Kalinsky KM M. Columbia University Medical Center, New York, NY; Food and Drug Administration; Mount Sinai School of Medicine, New York, NY; Albert Einstein College of Medicine, Bronx, NY and Weill Cornell Medical Center, New York, NY.

Body: Background

Preclinical and clinical studies suggest that trastuzumab resistance in HER2 amplified breast cancer (HER2+ BC) is mediated by cross-activation of alternative signaling pathways. Computational analysis and pooled whole-genome RNAi screens in HER2 transformed BC cell lines identified the IL6/JAK2/STAT3 axis as a master regulator pathway. The combination of trastuzumab plus ruxolitinib, a JAK1/JAK2 inhibitor, demonstrated synergistic tumor growth inhibition in mouse xenografts of HER2 transformed BC cell lines. These data provide the rationale for studying the efficacy of ruxolitinib and trastuzumab in a clinical trial.

Design

This is a multi-center, open-label, phase I/II trial of ruxolitinib plus trastuzumab in patients (pts) with HER2+ metastatic BC (MBC) who have progressed on ≥ 2 HER2-directed therapies in the metastatic setting (including trastuzumab, pertuzumab and T-DM1). The phase I is an adaptive design with 10 pts, using the time-to-event continual reassessment method to determine the recommended phase II dose. Phase II will be a non-randomized, open-label trial with 30 evaluable pts. The duration of a treatment cycle is 21 days, with trastuzumab given on Day 1 and ruxolitinib taken orally twice daily continuously. The primary endpoint of phase I is to determine the maximum tolerated dose of the drug combination. The phase I dose range for ruxolitinib is 10-25 mg BID (dose level 0: 20 mg BID). Response is assessed by imaging every 9 weeks. Blood samples and optional tissue biopsies are obtained for biomarker analysis at the following time points: pre-treatment, on-treatment C2D1, and at progression.

Results

Phase I started accrual in the fall of 2014. The trial has accrued 12 patients, with 9 evaluable and 3 non-evaluable patients. Of the evaluable patients, the mean age was 55.9 (range 32-69). Of these, 7 were postmenopausal (78%) 5/9 (56%) were estrogen receptor positive, and all had measurable disease. The mean number of prior lines of therapy in the metastatic setting was 5.6 (range: 3-8), including a mean of 3.2 (range: 2-5) prior regimens containing HER2 targeted therapies. As of 6/12/16, 2 patients remain on therapy. As this is an adaptive design, efficacy and drug tolerability will not be mentioned in this abstract to not bias the ongoing analysis. However, we anticipate that by SABCS 2016, 10 evaluable patients will have completed the DLT period – at which point, complete data will be presented.

Conclusion

Ruxolitinib plus trastuzumab is a novel, non-chemotherapy containing regimen. The phase I analysis is ongoing. We plan on reporting full safety/tolerability and efficacy data once 10 evaluable patients have completed the phase I (9/10 have currently completed DLT period). Given an early signal in HER2+ breast cancer, in this heavily pretreated population we will proceed directly to a phase II trial with the combination.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-38

Title: Cardiac safety of dual anti-HER2 therapy in the neoadjuvant setting for treatment of HER2-positive breast cancer

Yu AF F, Wang R, Singh J, Liu JE E, Eaton A, Jones L, Oeffinger K, Steingart RM M, Hudis CA A and Dang CT T. Memorial Sloan Kettering Cancer Center, New York, NY.

Body: Background: Trastuzumab and pertuzumab are approved for the treatment of HER2-positive breast cancer in the neoadjuvant setting. However, limited cardiac safety data is available when used in combination with doxorubicin-based chemotherapy. At a single center we retrospectively report the cardiac safety of dose-dense doxorubicin and cyclophosphamide (AC) followed by paclitaxel, trastuzumab, and pertuzumab (THP) in the neoadjuvant setting followed by adjuvant trastuzumab-based therapy.

Methods: Patients treated with neoadjuvant dose dense AC followed by THP from September 1, 2014 to March 1, 2015 were retrospectively identified. Patients then had breast surgery and completion of anti-HER2 therapy for 1 year. The primary outcome was cardiac event rate, defined by New York Heart Association (NYHA) class III/IV or cardiac death. The secondary outcome was significant asymptomatic decline in left ventricular ejection fraction (LVEF) of 10%-15% from baseline to below the lower limit of normal, or $\geq 16\%$ from baseline. Patients underwent LVEF monitoring at baseline prior to AC, prior to initiation of THP, and serially during 1 year of anti-HER2 therapy (neoadjuvant to adjuvant phases).

Results: Fifty-seven patients were included in the study. The median age was 46 years (range 26-68 years). The median number of cycles of trastuzumab and pertuzumab in the neoadjuvant setting was 6 (range 3-8) and 6 (range 2-8), respectively. In the adjuvant setting, 56 (98%) patients received trastuzumab with pertuzumab with a median cycle number of 12 (range 1-13). Two of 57 (3.5%) patients developed NYHA class III/IV heart failure after 5 and 9 months of trastuzumab, leading to permanent discontinuation of anti-HER2 treatment. Additionally, 7 (12.3%) patients developed a significant LVEF decline (without severe HF symptoms). The median LVEF was 65% (range 55-75%) at baseline and 64% (range 53-72%) after AC. There was a reduction in median LVEF to 60% (range 35-70%), 60% (range 23-73%), 61% (range 25-73%), and 58% (range 28-66%) at 3, 6, 9, and 12 months (+/- 6 weeks) of trastuzumab-based therapy.

Conclusion: In patients with HER2-positive breast cancer treated with neoadjuvant dose-dense AC and THP followed by completion of anti-HER2 therapy in adjuvant setting, the overall rate of NYHA Class III/IV heart failure was comparable to rates reported in trials of sequential doxorubicin and trastuzumab. Our findings do not suggest an increased risk of cardiotoxicity when pertuzumab is added to trastuzumab following a doxorubicin-based regimen.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-39

Title: Neo-adjuvant treatment with trastuzumab and pertuzumab associated with palbociclib and fulvestrant in HER2-positive and ER-positive breast cancer: Effect on Ki67 during and after treatment. A phase II Michelangelo study

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Body: Background: In metastatic breast cancer, the CDK4,6 inhibitor palbociclib associated with fulvestrant proved superior to fulvestrant alone in HER2-negative breast cancer (NEJM 2015). In HER2-positive tumors, the dual blockade of the receptor plus an aromatase inhibitor led to a 21% rate of pCR in a neoadjuvant setting in ER+ cancers (J Clin Oncol 2013). In preclinical studies concomitant inhibition of CDK4,6 and trastuzumab led to synergistic antitumor activity (Cancer Cell 2016). The extent of early change and the persistence of Ki67 down-regulation are robust markers of the effects of endocrine treatments in hormone receptors positive breast carcinomas in the setting of neoadjuvant endocrine therapy (Ann Oncol 2012).

Methods: In this exploratory Phase II trial (NCT02530424), women with invasive unilateral non metastatic ER-positive breast cancer expressing HER2 and suitable for neoadjuvant therapy were treated with every 3 wks trastuzumab and pertuzumab for 6 cycles combined with palbociclib 125 mg po q.d. x 21 q. 4 wks and fulvestrant i.m. 500 mg, both given for 5 cycles (HPPF). The primary endpoint was characterization of Ki67 changes from baseline before therapy, at 2 weeks and at surgery.

Results: A total of 23 patients with centrally confirmed HER2 and ER positive breast cancer were recruited for this study. Ki67 was also assessed centrally. At baseline 30% of cases were classified as locally advanced, 43% as cN0 and 78% had Ki67 values > 20%. Objective clinical response was documented in 96% of patients and pCR (absence of invasive cells in breast and axillary nodes) was documented in 22% (pCR in breast only was 26%). The geometric means of Ki67 expression assessed at baseline, after 2 weeks of treatment and at surgery were 30.8 (SD 15.1), 3.9 (SD 15.9) and 9.2 (SD 16.6) respectively. The mean change in Ki67 values from baseline to after 2 weeks was -24.5 (paired t-test: P < 0.0001) and -13.9 (P = 0.008) from baseline to surgery. Treatment was fairly well tolerated. No serious adverse events > grade 3 were reported. The most frequent G 3 adverse events were neutropenia (26% of patients) and gastrointestinal disorders (17%).

Conclusions: Neoadjuvant triple targeting of ER, HER2 and Rb in HER2+/ER+ breast cancer treatment caused a significant and rapid decrease of Ki67 that was of larger magnitude after 2 weeks than at surgery irrespective of the recorded objective clinical response. The good tolerability, the rapid effect on Ki67, the consistent clinical response, and the rate of pCR with this chemotherapy-free approach support further clinical testing and additional molecular characterization.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-40

Title: *In vitro* and *in vivo* activity of HER2-targeted antibody-liposomal doxorubicin conjugate MM-302 in HER2-intermediate tumors

Geretti E, Espelin C, Adiwijaya B, Coma S, Koncki Z, Sumner P, Dumont N, Garcia G, Bloom T, Janovsky J, Reynolds J, Campbell K, Moyo V, Molnar I, LoRusso P, Krop I, Miller K, Ma C, Munster P and Wickham T. Merrimack Pharmaceuticals Inc., Cambridge, MA; Yale Cancer Center, New Haven, CT; Dana-Farber Cancer Institute, Boston, MA; Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN; Washington University School of Medicine, St. Louis, MO and Helen Diller Family Comprehensive Cancer Center, San Francisco, CA.

Body: Background: MM-302 is a HER2-targeted antibody–liposomal doxorubicin conjugate designed to target doxorubicin to HER2-expressing tumor cells. MM-302 showed an acceptable safety profile and promising activity in a Phase 1 study in HER2-positive (IHC 3+ or 2+ and *HER2* FISH amplified) metastatic breast cancer (NCT01304797), and it is now being evaluated in a Phase 2 trial in the same setting (NCT02213744). A substantial percentage (~30%) of breast cancer patients show positive HER2 IHC (1+/2+) without *HER2* gene amplification (“HER2 intermediate”). This population is not eligible for treatment with currently approved HER2-targeted therapies. The goal of this work is to evaluate preclinical activity of MM-302 in HER2 intermediate models.

Methods: *In vitro* binding and viability studies were performed with MM-302, PEGylated liposomal doxorubicin (PLD) and T-DM1 in a panel of cell lines at different HER2 expression. MM-302, PLD and T-DM1 cellular uptake was investigated *in vivo* in IHC 0, 1+, 2+ and 3+ tumor models. Anti-tumor activity of MM-302 relative to PLD and T-DM1 was evaluated in the HER2 IHC 1+ FISH NEG models T47D (breast metastatic) and IGROV1 (ovarian). HER2-mediated tumor cell uptake of MM-302 was also evaluated in frozen patient biopsies collected 3 days post infusion of MM-302 (8-50 mg/m²; alone or in combination with trastuzumab, with or without cyclophosphamide) (Phase 1 study NCT01304797).

Results: MM-302 efficiently bound to and induced tumor cell death across a panel of cell lines at different HER2 expression. *In vivo* evaluation showed increased HER2-mediated uptake of MM-302 over PLD in tumor cells in IHC 1+, 2+, and 3+ tumors but not in IHC 0 tumors. As expected, uptake of T-DM1 into HER2 intermediate models was negligible, and T-DM1 mainly localized in the periphery of the tumors and within the stromal cells. Treatment with MM-302 resulted in increased anti-tumor activity in the IGROV1 xenograft model, and in a significantly higher inhibition of T47D metastatic spread to lungs, ovaries, adrenal glands and liver relative to both PLD and T-DM1.

The preclinical findings were in line with observations on post-treatment biopsies from MM-302 Phase 1 study NCT01304797. HER2 expression within individual samples was found to be heterogeneous, ranging from ~1 x 10⁵ to over 1 x 10⁶ HER2 receptors per cell. Evaluation of MM-302 uptake in tumor cells at different HER2 levels within the same sample revealed that the magnitude of MM-302 tumor cell uptake was comparable across the range of HER2 expression from ~1 X 10⁵ to over 1 X 10⁶ HER2 receptors per cell.

Conclusions: Our data suggest that MM-302 effectively targets tumor cells expressing various levels of HER2 in preclinical models as well as in patient tumors. Hence, in addition to investigating activity in HER2-positive patients, MM-302 development may be extended to patients with intermediate HER2 expression (HER2 IHC 1+/2+, FISH-negative), who are ineligible to currently approved HER2-targeted therapies, and who represent a significant unmet medical need.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-41

Title: Primary analysis of BERENICE: A phase II cardiac safety study of pertuzumab, trastuzumab, and neoadjuvant anthracycline-based chemotherapy in patients with locally advanced, inflammatory, or early-stage, unilateral, and invasive HER2-positive breast cancer

Swain SM M, Ewer MS S, Viale G, Delaloge S, Ferrero JM, Verrill M, Colomer R, Vieira C, Werner TL L, Douthwaite H, Bradley D, Waldron-Lynch M, Eng-Wong J and Dang C. Georgetown University Medical Center, Washington, DC; The University of Texas MD Anderson Cancer Center, Houston, TX; European Institute of Oncology, University of Milan, Milan, Italy; Institut Gustave Roussy, Paris, France; Centre Antoine Lacassagne, Nice, France; Northern Centre for Cancer Care, Newcastle upon Tyne, United Kingdom; Hospital Universitario La Princesa, Madrid, Spain; Instituto Português de Oncologia Francisco Gentil (IPOFG), Porto, Portugal; Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; Roche Products Ltd, Welwyn Garden City, United Kingdom; Clinical Science, Global Product Development, Welwyn Garden City, United Kingdom; Product Development – Oncology, Genentech, Inc., South San Francisco, CA and Memorial Sloan-Kettering Cancer Center, New York, NY.

Body: Background: Neoadjuvant pertuzumab (P)+trastuzumab (H)+standard chemotherapy (CT) significantly increases pathologic complete response (pCR) rates v H+CT. However, anti-HER2 therapy following anthracyclines can cause heart failure, and data are limited or lacking on combining P+H after epirubicin or doxorubicin. We report cardiac and overall safety as well as total pCR (tpCR) rates with widely used but understudied anthracycline-containing regimens.

Methods: BERENICE (NCT02132949), a non-randomized, open-label, phase II study, enrolled patients (pts) with centrally confirmed HER2-positive, locally advanced, inflammatory, or early-stage, unilateral, and invasive breast cancer, Eastern Cooperative Oncology Group performance status ≤ 1 , and baseline left ventricular ejection fraction (LVEF) $\geq 55\%$. In the neoadjuvant period, Cohort A pts received four q2w dose-dense doxorubicin+cyclophosphamide cycles (60 mg/m²/600 mg/m² with granulocyte-colony stimulating factor support as needed) followed by 12 qw paclitaxel doses (80 mg/m²) + four q3w P+H cycles (P 840 mg, then 420 mg; H 8mg/kg, then 6 mg/kg). Cohort B received four q3w fluorouracil/epirubicin/cyclophosphamide cycles (500 mg/m²/100 mg/m²/600 mg/m²) followed by four q3w docetaxel cycles (75 mg/m² escalated to 100 mg/m²) + four q3w P+H cycles. Surgery was performed after cycle 8 for both cohorts. The primary objective was to evaluate cardiac safety during the neoadjuvant period, assessed by incidence of 1) New York Heart Association (NYHA) Class III/IV heart failure and 2) significant LVEF declines ($\geq 10\%$ from baseline with a value of $< 50\%$). Secondary objectives included assessments of adverse event (AE) rates and tpCR (ypT0/Tis ypN0; NX was considered non-tpCR). Results are descriptive; no statistical hypothesis testing was planned.

Results: Four hundred one pts were enrolled between Jul 2014–Aug 2015. Clinical cutoff was Mar 3, 2016. Demographics and baseline characteristics were generally balanced between cohorts; 64.3% v 61.7% of pts had centrally confirmed hormone receptor (HR)-positive disease and 95.0% v 93.0% had T1–T3 primary tumors.

Three pts in Cohort A and none in Cohort B experienced NYHA Class III/IV heart failure during neoadjuvant treatment (table). Thirteen pts in Cohort A v four in Cohort B had significant LVEF declines (table). One Cohort B pt's LVEF decline was prior to anti-HER2 treatment.

AE and serious AE (SAE) rates were well balanced between cohorts. The most common AEs were nausea, diarrhea, and alopecia. The most common SAE was febrile neutropenia.

tpCR rates were similar between cohorts (table).

Neoadjuvant period	Cohort A	Cohort B
Safety: Pts, n (%)	N=199	N=198
NYHA Class III/IV heart failure	3 (1.5%)	0
Significant LVEF decline	13 (6.5%)	4 (2.0%)
tpCR (ypT0/Tis ypN0): Pts, n/N (%)		

Intention-to-treat	123/199 (61.8%)	122/201 (60.7%)
HR-positive	66/128 (51.6%)	71/124 (57.3%)
HR-negative	53/65 (81.5%)	51/75 (68.0%)

Conclusion:

Cardiac and general safety of the two anthracycline-containing regimens in BERENICE were as expected and were consistent with the known P+H+CT profiles. Both regimens were active, and tpCR rates were high.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-21-42

Title: MetaPHER phase IIIb multicenter, open-label, single-arm safety study of subcutaneous trastuzumab in combination with pertuzumab and docetaxel in patients with HER2-positive advanced breast cancer: First results

Kümmel S, Abraham J, Martin M, Crepelle-Fléchais A, Swat A, Nüesch E, Shing M and Tondini CA A. Kliniken Essen-Mitte, Essen, Germany; Velindre Cancer Centre, Cardiff, United Kingdom; Hospital General Universitario Gregorio Marañón, Madrid, Spain; Global Product Development, F. Hoffman-La Roche Ltd, Basel, Switzerland; Global Product Development Biometrics, F. Hoffman-La Roche Ltd, Basel, Switzerland; Global Pharma Development, Genentech, Inc, South San Francisco, CA and ASST Papa Giovanni XXIII, Bergamo, Italy.

Body: Background: MetaPHER investigates fixed-dose subcutaneous trastuzumab (Herceptin® [H SC]) in combination with intravenous (IV) pertuzumab (PERJETA® [P IV]) plus docetaxel (D IV). The MetaPHER study treatment regimen is based on CLEOPATRA (phase III; NCT00567190), which demonstrated a significant progression-free and overall survival benefit in patients (pts) with HER2-positive metastatic breast cancer (BC) following first-line treatment with IV trastuzumab (Herceptin® [H IV]) plus P IV plus D IV. Fixed-dose H SC was non-inferior to H IV in terms of pathological complete response and serum trough concentration, and had a comparable safety profile to H IV, in pts with HER2-positive early BC in Hannah (phase III; NCT00950300). Furthermore, PrefHer (NCT01401166) results showed compelling patient preference for H SC vs H IV (88.9% vs 9.6%) in the HER2-positive early BC setting. To date, limited data exist for H SC in combination with P IV and D IV; we report the first results of safety and tolerability from MetaPHER for this combination in pts with HER2-positive advanced BC.

Methods: MetaPHER enrollment is ongoing at >100 sites in 12 countries. Females aged ≥18 years with metastatic or locally recurrent HER2-positive BC previously untreated with non-hormonal anticancer therapy, an Eastern Cooperative Oncology Group performance status of 0 or 1, and left ventricular ejection fraction (LVEF) ≥50% receive fixed-dose H SC 600 mg/5 mL q3w, P IV (840 mg loading dose, then 420 mg at subsequent cycles q3w), and D IV (at least 6 cycles with a recommended initial dose of 75 mg/m² q3w; possible escalation to 100 mg/m² q3w and continuation of D IV after Cycle 6 at the investigator's discretion). Study treatment is given until disease progression, unacceptable toxicity, withdrawal of consent, death, or predefined study end, whichever occurs first. The primary endpoint is safety and tolerability of H SC in combination with P IV and D IV in pts with HER2-positive advanced BC. Adverse events (AEs) and serious AEs (SAEs) were graded per National Cancer Institute – Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0. Results are descriptive.

Results: 150 pts were enrolled and received a median of 4 treatment cycles by the data cutoff date of June 23, 2016. Safety data are shown in the table.

Pts with ≥1 event, n (%)	H SC + P IV + D IV (N = 150)
Any grade AEs	136 (90.7)
Grade ≥3 AEs	59 (39.3)
SAEs	23 (15.3)
Deaths	2 (1.3)
AEs leading to discontinuation of H SC, P IV, or D IV	16 (10.7)
AEs leading to discontinuation of H SC	5 (3.3)
AEs of Interest	
Administration-related and local injection-site reactions for H SC	5 (3.3)*
<i>Cardiac:</i>	
Grade ≥3 cardiac AEs [†]	1 (0.7)
Any grade congestive heart failure [‡]	0
Pts with a significant LVEF drop [§]	1 (0.7)

*Grade 1 & 2 AEs only; †Events classified as System Organ Class Cardiac Disorders; ‡Preferred terms of reported AEs; §≥10% from baseline to LVEF <50%.

Conclusion: MetaPHER's first report of H SC with P IV and D IV as first-line treatment for pts with HER2-positive advanced BC is consistent with the known safety profile for H IV with P IV and D IV from CLEOPATRA, and no new safety signals were identified.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-02

Title: Evaluation of veliparib (V) and temozolomide (TMZ) in a phase 2 randomized study of the efficacy and tolerability of V+TMZ or carboplatin (C) and paclitaxel (P) vs placebo (Plc)+C/P in patients (pts) with *BRCA1* or *BRCA2* mutations and metastatic breast cancer

Diéras V, Han HS, Robson ME E, Palácová M, Marcom PK Kelly, Jager A, Bondarenko I, Citrin D, Campone M, Telli ML L, Domchek SM M, Friedlander M, Kaufman B, Ratajczak C, Coates A, Bonnet P, Qin Q, Qian J, Giranda VL L, Shepherd SP P, Puhalla S and Isakoff SJ J. Institut Curie, Paris, France; Moffitt Cancer Center, Tampa, FL; Memorial Sloan Kettering Cancer Center, New York, NY; Masarykův Onkologický ústav, Brno, Czech Republic; Duke University, Durham, NC; Erasmus MC Cancer Institute, Rotterdam, Netherlands; Dnepropetrovsk City Hospital, Dnepropetrovsk, Ukraine; Midwestern Regional Medical Center, Zion, IL; Institut de Cancérologie de l'Ouest, Saint Herblain, France; Stanford University School of Medicine, Stanford, CA; University of Pennsylvania, Philadelphia, PA; Prince of Wales Hospital, Sydney, NSW, Australia; Sheba Medical Center, Tel Hashomer, Israel; AbbVie, Inc, Chicago, IL; University of Pittsburgh Cancer Institute, Pittsburgh, PA and Massachusetts General Hospital, Boston, MA.

Body: Background: V is a potent, poly(ADP-ribose) polymerase (PARP) inhibitor that obstructs DNA damage repair. *BRCA1/2* tumors are defective in homologous recombination, which leads to more error-prone mechanisms of DNA repair and increased sensitivity to PARP inhibition. V enhances the antitumor activity of alkylating agents such as TMZ in preclinical models. In addition, V+TMZ showed promising activity in a single-arm phase 2 study in pts with *BRCA1/2* mutations. This phase 2 trial (NCT01506609) investigated the efficacy and tolerability of V+TMZ (or V+C/P) compared to Plc+C/P in pts with locally recurrent or metastatic breast cancer harboring a *BRCA1* or *BRCA2* mutation. Results from the primary analysis for the V+TMZ arm vs Plc+C/P are presented, and the V+C/P vs Plc+C/P results will be presented separately.

Methods: Male and female pts aged ≥ 18 years with histologically confirmed locally recurrent or metastatic breast cancer were randomized 1:1:1 to: 1) V 40 mg BID D1–7 + TMZ 150–200 mg/m² QD D1–5, 28-D cycle; 2) V 120 mg BID D1–7 + C AUC 6, D3 and P 175 mg/m², D3, 21-D cycle; 3) placebo BID D1–7 + C/P. Key eligibility criteria included known deleterious *BRCA1/2* mutation, ≤ 2 prior chemotherapies for metastatic disease, no prior platinum agent, and no CNS metastases. Randomization was stratified by hormone receptor status, prior cytotoxic therapy (yes vs no), and ECOG PS (0–1 vs 2). The primary endpoint was progression-free survival (PFS) per RECIST 1.1 by independent review. Overall survival (OS), objective response rate (ORR), and safety/tolerability were also evaluated.

Results: A total of 290 pts (284 *BRCA+* per central lab) were randomized (V+TMZ, n=94 [91 *BRCA+*]). Baseline demographics and disease characteristics were comparable among treatment arms; 41.3% of pts had triple-negative breast cancer (TNBC) and 31.7% had received >2 prior regimens. Median study drug exposure was 6 cycles for the V+TMZ arm and 10 cycles for the Plc+C/P arm. Median PFS, median OS (interim), and ORR for V+TMZ were inferior to Plc+C/P (PFS 7.4 vs 12.3 mo, OS 19.1 vs 25.0 mo, and ORR 28.6% vs 61.3%). In pts with TNBC, median PFS was 5.5 (3.1–8.5) mo; 8.4 (6.8–10.6) mo for pts with non-TNBC. Treatment-emergent adverse events (AEs) of interest occurring differentially with V+TMZ are shown in Table 1. Grade ≥ 3 AEs in $\geq 30\%$ of pts in the V+TMZ arm were thrombocytopenia (48%) and neutropenia (37%).

Conclusions: V+TMZ provided durable responses, with less neutropenia, alopecia, and neuropathy than Plc+C/P; however, PFS, OS, and ORR were inferior in the TMZ arm compared to C/P.

Table 1

Treatment-Emergent AEs, n (%)	V+TMZ, n=93	Plc+C/P, n=96
Neutropenia	46 (50)	71 (74)
Alopecia	10 (11)	55 (57)
Peripheral neuropathy	11 (12)	56 (58)
Thrombocytopenia	73 (79)	67 (70)
Nausea	70 (75)	56 (58)

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-03

Title: Palbociclib in combination with endocrine therapy in treatment-naive and previously treated elderly women with HR+, HER2– advanced breast cancer: a pooled analysis from randomized phase 2 and 3 studies

Rugo HS S, Turner NC C, Finn RS S, Joy AA A, Verma S, Harbeck N, Moulder S, Masuda N, Im Y-H, Zhang K, Kim S, Sun W, Schnell P, Huang-Bartlett C and Slamon D. University of California San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; Institute of Cancer Research and Royal Marsden Hospital, London, United Kingdom; David Geffen School of Medicine, Los Angeles, CA; Cross Cancer Institute, University of Alberta, Edmonton, AB, Canada; University of Calgary, AB, Canada; Brustzentrum der Universität München (LMU), Munich, Germany; University of Texas, MD Anderson Cancer Center, Houston, TX; National Hospital Organization Osaka National Hospital, Osaka, Japan; Sungkyunkwan University School of Medicine, Seoul, Republic of Korea; Pfizer Inc, San Diego, CA; Pfizer Oncology, La Jolla, CA; Pfizer Inc, New York, NY and Pfizer Inc, Collegetown, PA.

Body: Background: At least 40% of breast cancers are diagnosed in women ≥ 65 y old and most are hormone receptor-positive/human epidermal growth factor receptor 2-negative (HR+/HER2-). Palbociclib (PAL) is an oral, small-molecule inhibitor of cyclin-dependent kinases 4 and 6. Randomized studies of PAL combined with endocrine therapy (ET) demonstrated significantly improved progression-free survival (PFS) in patients (pts) with treatment-naive and previously treated advanced breast cancer (ABC).

Methods: We evaluated the efficacy of PAL+ET vs ET alone in pts aged $\geq 65-74$ and ≥ 75 y across multiple pivotal randomized phase 2 and 3 studies. Safety and pharmacokinetics (PK) data (blood samples collected from pts in phase 1/2 [PALOMA-1] and phase 1 studies [NCT00141297 and NCT00420056]) for PAL+ET were pooled and compared across age groups. Pts who had not received treatment for ABC were randomized to receive PAL+letrozole (LET) or LET alone/with placebo (PBO; PALOMA-1, open-label/PALOMA-2, double-blind). Pts who had progressed on prior ET were randomized to receive PAL+fulvestrant (FUL) or PBO+FUL (PALOMA-3, double-blind). The primary endpoint for these studies was investigator-assessed PFS. Safety assessments and blood counts occurred at baseline and every 2 weeks for the first 2 cycles and on day 1 of subsequent cycles.

Results: Among 872 pts treated with PAL+ET, 221 (25%) were aged $\geq 65-74$ y and 83 (10%) were ≥ 75 y (PAL+LET: n=528, 162 and 56, respectively; PAL+FUL: n=347, 59 and 27). Median (range) treatment durations were 440 (1-1615) d, 502 (1-1615) d, and 459 (21-1404) d, respectively. Improvement in efficacy endpoints was seen with PAL+ET vs ET across all age groups (**Table 1**). Incidence of adverse events (AEs), serious AEs and discontinuations due to AEs were similar in the overall population (99%, 19%, 3%) and in pts aged $\geq 65-74$ (99%, 25%, 5%) and ≥ 75 y (100%, 30%, 6%). Incidence of all grades and grade 3/4 neutropenia were also similar across age groups (overall: 67% and 54%; $\geq 65-74$ y: 64% and 51%; ≥ 75 y: 77% and 60%). PK analysis showed no clinically relevant differences between arithmetic means, medians, and geometric means of the apparent oral clearance across age groups.

Conclusions: PAL in combination with ET is an effective and well-tolerated treatment option for elderly pts with HR+/HER2- endocrine-sensitive and -resistant ABC. A dose adjustment based on age is not required.

Sponsor: Pfizer

Table 1. PFS in pts $\geq 65-74$ and ≥ 75 y (ITT populations)

	Overall	Aged $\geq 65-74$ y	Aged ≥ 75 y
PALOMA-1/PALOMA-2			
PAL+LET vs LET/LET+PBO,* n	528 vs 303	162 vs 94	
HR (95% CI); 1-sided P value	0.53 (0.44-0.64); <0.0001	0.66 (0.45-0.97); 0.0162	0.31 (0.16-0.61); 0.0002
Median PFS (95% CI), mo	24.4 (22.0-26.2) vs 13.6 (11.1-16.4)	27.5 (24.2-NR) vs 21.8 (16.3-31.3)	NR (19.2-NR) vs 10.9 (4.9-24.9)

PALOMA-3			
PAL+FUL vs	347 vs 174	59 vs 37	27 vs 6
FUL+PBO, n			
HR (95% CI);	0.46 (0.36-0.59);	0.25 (0.14-0.45);	0.87 (0.27-2.79);
1-sided <i>P</i> value	<0.0001	<0.0001	0.4074
Median PFS (95% CI), mo	9.5 (9.2-11.0) vs	16.1 (12.0-NR) vs	13.6 (7.5-NR) vs
	4.6 (3.5-5.6)	3.7 (1.9-5.3)	7.4 (1.9-NR)

HR=hazard ratio; ITT=intent to treat; NR=not reached. *Does not include 5 pts from phase 1 of PALOMA-1.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-04

Title: A randomized, double-blind, phase 2 study of ruxolitinib (RUX) or placebo (PBO) in combination with capecitabine (CAPE) in patients (pts) with advanced HER2-negative breast cancer (ABC) and elevated C-reactive protein (CRP), a marker of systemic inflammation

O'Shaughnessy J, DeMichele A, Ma C, Richards P, Yardley DA A, Wright G, Kalinsky K, Steis R, Diab S, Kennealey G, Geschwindt R, Jiang W and Rugo H. Baylor University Medical Center, Texas Oncology, US Oncology, Dallas, TX; University of Pennsylvania, Philadelphia, PA; Washington University School of Medicine, St Louis, MO; Oncology & Hematology Associates of Southwest Virginia, Inc, Salem, VA; Sarah Cannon Research Institute and Tennessee Oncology PLLC, Nashville, TN; Florida Cancer Specialists, St Petersburg, FL; Columbia University Medical Center, New York, NY; Northside Hospital, Inc, Atlanta, GA; Rocky Mountain Cancer Centers, Aurora, CO; Incyte Corporation, Wilmington, DE and University of California, San Francisco, CA.

Body: Background: Systemic inflammation is associated with poor prognosis in pts with ABC. The JAK/STAT pathway is a key regulator of inflammatory signaling, associated with tumorigenesis, cell survival, and progression. We evaluated the efficacy and safety of RUX, a JAK1/JAK2 inhibitor, plus CAPE in pts with HER2-negative ABC and high systemic inflammation defined by the modified Glasgow Prognostic Score (mGPS). **Methods:** In this double-blind phase 2 trial, pts were randomized 1:1 to 21 day cycles of RUX+CAPE or PBO+CAPE: RUX 15 mg or PBO PO BID for 21 d; CAPE 1000 mg/m² PO BID for 14 d. Key inclusion criteria were systemic inflammation by mGPS of 1 or 2 (ie, CRP >10 mg/L), ECOG performance status ≤2, ≤2 prior chemotherapy regimens, and no prior CAPE. The primary endpoint was overall survival (OS); key secondary endpoints were progression-free survival (PFS), objective response rate (ORR; complete [CR] + partial response [PR]) per RECIST v1.1, clinical benefit rate (CBR; CR + PR + stable disease for ≥6 mo), duration of response, and safety. Treatment differences in OS and PFS were analyzed by the log-rank test; HRs and CIs were analyzed by the Cox proportional hazards model. **Results:** Baseline characteristics were similar between pts randomized to RUX+CAPE (n=76) vs PBO+CAPE (n=73): mGPS status (1, 82.9% vs 83.6%), hormone receptor (HR) status (positive, 67.1% vs 63.0%), and number of prior chemotherapy regimens for ABC (0, 50.0% vs 50.7%; 1, 38.2% vs 34.2%; 2, 9.2% vs 13.7%). Median treatment durations were 85 d with RUX in the RUX+CAPE group and 65 d with PBO in the PBO+CAPE group. Median OS was 11.2 mo with RUX+CAPE vs 10.9 mo with PBO+CAPE (HR, 0.932; 95% CI, 0.59–1.46; *P*=0.762). Median OS was 6.1 mo with RUX+CAPE vs 5.5 mo with PBO+CAPE in HR-negative pts and 11.7 mo and 12.2 mo in HR-positive pts. Median PFS was 4.5 mo with RUX+CAPE and 2.5 mo with PBO+CAPE (HR, 0.737; 95% CI, 0.49–1.12; *P*=0.151). Median PFS was 2.1 mo with RUX+CAPE vs 2.2 mo with PBO+CAPE in HR-negative pts and 6.1 mo and 4.1 mo in HR-positive pts. ORRs were 28.9% and 13.7% (*P*=0.024) in the RUX+CAPE and PBO+CAPE arms, respectively. The CBRs were 13.2% and 6.8%, respectively (*P*=0.278). Worsening of hematologic toxicity was higher and rates of grade 3/4 palmar-plantar erythrodysesthesia (PPE) were lower (1.4% vs 12.7%, respectively) with RUX+CAPE (**Table**).

Safety

	RUX+CAPE (n=71)		PBO+CAPE (n=71)	
%	All-Grade	Grade 3/4	All-Grade	Grade 3/4
Nonhematologic Adverse Event*				
Fatigue	56.3	5.6	43.7	4.2
Nausea	54.9	8.5	49.3	5.6
Diarrhea	47.9	8.5	26.8	2.8
PPE	46.5	1.4	38.0	12.7
Vomiting	38.0	5.6	29.6	4.2
Hypokalemia	15.5	8.5	7.0	2.8
Worsening of Hematologic Toxicity†				
Anemia	80.3	23.9	56.3	7.0

Lymphopenia	40.8	15.5	45.1	12.7
Neutropenia	39.4	11.3	22.5	2.8
Thrombocytopenia	39.4	11.3	15.5	1.4

*Most common all-grade ($\geq 35\%$) or grade 3/4 ($\geq 5\%$) events in the RUX+CAPE arm (safety group). †Laboratory abnormalities.

Conclusion: These data support the prognostic capabilities of the mGPS. The addition of RUX to CAPE for pts with ABC and high systemic inflammation was associated with an improved ORR compared with PBO+CAPE, but did not improve OS or PFS.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-05

Title: First-line ribociclib plus letrozole in patients with *de novo* HR+, HER2– advanced breast cancer (ABC): A subgroup analysis of the MONALEESA-2 trial

O'Shaughnessy J, Petrakova K, Sonke GS S, André F, Conte P, Arteaga CL L, Cameron DA A, Hart LL L, Villanueva C, Jakobsen EH H, Lindquist D, Souami F, Li X, Germa C, Hirawat S and Hortobagyi GN N. Texas Oncology-Baylor Charles A. Sammons Cancer Center and US Oncology Network, Dallas, TX; Masaryk Memorial Cancer Institute, Brno, Czech Republic; Netherlands Cancer Institute and BOOG Study Center, Amsterdam, Netherlands; Gustave Roussy, Villejuif, France; University of Padova and Istituto Oncologico Veneto, IRCCS, Padova, Italy; Vanderbilt-Ingram Cancer Center, Nashville, TN; Edinburgh Cancer Research Centre, University of Edinburgh, Edinburgh, United Kingdom; Florida Cancer Specialists/Sarah Cannon Research Institute, Fort Myers, FL; University Hospital of Besançon, Hospital Jean Minjot, Besançon, France; Lillebaelt Hospital, Vejle, Denmark; Arizona Oncology, US Oncology Network, Sedona, AZ; Novartis Pharma AG, Basel, Switzerland; Novartis Pharmaceuticals Corporation, East Hanover, NJ and University of Texas M.D. Anderson Cancer Center, Houston, TX.

Body: Background: Around 15,000 US patients per year are diagnosed with *de novo* ABC. Due to the absence of prior systemic treatment for breast cancer, tumors of patients with *de novo* ABC may exhibit a different disease biology, which could result in different tumor responses compared with patients who have relapsed breast cancer. Ribociclib is an orally bioavailable cyclin-dependent kinase (CDK) 4/6 inhibitor. Results from MONALEESA-2, a double-blind, placebo-controlled, randomized Phase 3 trial (NCT01958021), demonstrated that first-line therapy with ribociclib + letrozole significantly improved progression-free survival (PFS) vs placebo + letrozole in patients with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2–) ABC. Many patients with *de novo* ABC receive endocrine therapy in the first line and in subsequent lines; here we present results from the MONALEESA-2 study in a subpopulation of patients with *de novo* ABC.

Methods: Postmenopausal women (N=668) with HR+, HER2– ABC who had no prior systemic therapy for ABC were randomized 1:1 (stratified by liver and/or lung metastases) to receive ribociclib (600 mg/day; 3-weeks-on/1-week-off) + letrozole (2.5 mg/day; continuous) or placebo + letrozole. Patients with *de novo* ABC were eligible. Additional eligibility criteria included measurable disease or ≥ 1 predominantly lytic bone lesion, Eastern Cooperative Oncology Group performance status ≤ 1 , and adequate bone marrow/organ function. Prior CDK4/6 inhibitors or systemic therapy for ABC were prohibited. Patients may have received ≤ 14 days of letrozole or anastrozole for ABC. The primary endpoint was locally assessed PFS; a predefined subgroup analysis evaluated PFS in patients with *de novo* ABC.

Results: In total, 227 patients with *de novo* ABC were enrolled. Patients with *de novo* ABC were equally distributed with 114 (34%) and 113 (34%) in the ribociclib + letrozole and placebo + letrozole arms, respectively. Median duration of exposure to study treatment in the ribociclib + letrozole vs placebo + letrozole arms was 14.1 vs 12.8 months. Treatment was discontinued in 84 (37%) patients with *de novo* ABC (ribociclib + letrozole vs placebo + letrozole, n [%]; 34 [30%] vs 50 [44%]). Reasons for treatment discontinuation (ribociclib + letrozole vs placebo + letrozole, n [%]) included disease progression (21 [18%] vs 41 [36%]), patient/physician decision (5 [4%] vs 6 [5%]), and adverse events (6 [5%] vs 3 [3%]). PFS was increased in patients with *de novo* ABC who received ribociclib + letrozole vs placebo + letrozole (hazard ratio=0.448 [95% confidence interval: 0.267–0.750]). The 12-month PFS event-free probability in patients with *de novo* ABC was 82% in the ribociclib + letrozole arm vs 66% in the placebo + letrozole arm.

Conclusions: The combination of ribociclib + letrozole significantly improved PFS compared with placebo + letrozole in postmenopausal women with HR+, HER2– *de novo* ABC at diagnosis and therefore may become an important treatment option in the *de novo* ABC setting.

Keywords: Advanced breast cancer; CDK4/6 inhibitor; Letrozole; Ribociclib

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-06

Title: Treatment postprogression in women with endocrine-resistant HR+/HER2- advanced breast cancer who received palbociclib plus fulvestrant in PALOMA-3

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Body: Background: Palbociclib (PAL) plus fulvestrant (FUL) demonstrated significant improvement in progression-free survival (PFS) vs placebo (PBO) plus FUL in women with HR+/HER2- advanced breast cancer (ABC) whose disease had progressed on prior endocrine therapy (ET). Because the effectiveness of standard therapies after progression on PAL is unknown, it is important to understand whether the benefits of PAL with respect to PFS extend to subsequent treatments.

Method: Pre-, peri-, and postmenopausal patients (pts) with HR+/HER2- ABC who had relapsed/progressed on prior ET were randomized 2:1 to PAL (125 mg/d oral [3 wk on drug, 1 wk off]) + FUL (500 mg/mo intramuscular, per standard of care) or PBO+FUL. One prior line of chemotherapy (CT) for ABC was allowed. For 9 mo immediately after participation in PALOMA-3, pts were assessed every 3 mo for information on poststudy progression and treatment; the type of treatment, its duration, and sites of progression were analyzed.

Results: As of Oct 2015, with a median follow-up of 15.8 mo for PAL+FUL and 15.3 mo for PBO+FUL, 333 PFS events were observed (200 and 133, respectively). Median PFS was 11.2 vs 4.6 mo (hazard ratio [HR], 0.497 [95% CI, 0.398–0.620]; $P<0.0001$). In both treatment arms, the most common sites of disease progression were the liver (149 [43%] and 94 [54%]) and bone (55 [16%] and 43 [25%]). 1 pt in each treatment arm developed new brain metastasis. The most commonly used postprogression regimens were capecitabine (n=57 [28.8%]), paclitaxel (n=22 [11.1%]), and exemestane (n=34 [17.2%]). Median time to subsequent CT (from randomization to the start of the first CT treatment poststudy) was longer with PAL+FUL (252 d) than with PBO+FUL (132 d). This was also observed in pts with visceral disease who never received CT in the advanced setting at study entry (median 208 and 125 d, respectively). 252 pts had subsequent disease progression, treatment discontinuation on immediate subsequent therapy, or died. Proportionally fewer pts in the PAL+FUL vs PBO+FUL arm discontinued next-line treatment (33% vs 46%), indicating that PAL does not adversely affect the efficacy of subsequent treatments. Analysis of the time to end of next-line treatment showed that the HR between the 2 treatment arms was 0.623 ([95% CI, 0.482–0.805]; 1-sided P value=0.0001) in favor of PAL treatment (**Table 1**).

Conclusion: The current analysis demonstrated that the treatment effect of PAL+FUL was retained in the most immediate line postprogression and that progression after PAL has no effect on the therapeutic benefit from subsequent treatments.

Sponsor: Pfizer

Table 1.

	PAL+FUL (n=347)	PBO+FUL (n=174)
Pts who progressed and were eligible for subsequent therapy, n (%)	198 (57)	130 (75)
Pt with postprogression event, n (%)	156 (45)	96 (55)
Objective progression on next-line therapy, n (%)	15 (4)	5 (3)
Deaths, n (%)	28 (8)	11 (6)
End of next-line therapy, n (%)	113 (33)	80 (46)

Median (95% CI) probability of being event-free at 12 mo	69.2 (64.0–73.8)	54.4 (46.4–61.7)
Median (95% CI) time to end of next-line therapy, mo	17.9 (16.0–NR)	12.8 (11.0–14.6)
Hazard ratio (95% CI)	0.623 (0.482–0.805)	
<i>P</i> value	0.0001	

FUL=fulvestrant; NR=not reached; PAL=palbociclib; PBO=placebo.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-07

Title: Long-term safety of palbociclib in combination with endocrine therapy in treatment-naive and previously treated women with HR+ HER2– advanced breast cancer: A pooled analysis from randomized phase 2 and 3 studies

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Body: Background: Palbociclib (PAL) is a selective and reversible oral cyclin-dependent kinase 4 and 6 inhibitor. Large randomized phase (ph) 2 and 3 trials showed significant improvement in progression-free survival (PFS) when PAL was combined with endocrine therapy (ET) vs ET alone in treatment (trt)-naive and previously treated hormone receptor–positive human epidermal growth factor receptor 2–negative (HR+ HER2–) advanced breast cancer (ABC) patients (pts). The median PFS with PAL+ET is >2 years as a first-line therapy for ABC and 11.2 mo in endocrine-resistant ABC. We evaluated the long-term safety in PALOMA-1, -2, and -3.

Methods: We analyzed the tolerability of PAL in combination with ET in 3 randomized trials. Pts untreated for ABC were randomized to receive PAL+letrozole (LET) vs LET alone in PALOMA-1 (ph 2, open-label; 1:1) or randomized to receive PAL+LET vs placebo (PBO)+LET in PALOMA-2 (ph 3, double-blind; 2:1). PALOMA-3 included pts who progressed on prior ET, randomized to receive PAL+fulvestrant (FUL) or PBO+FUL (ph 3, double-blind; 2:1). Safety assessments, including a complete blood count, were done at baseline, on D1 of each cycle, and on D14 of the first 2 cycles. We evaluated adverse events (AEs) by 6-mo intervals (out to 36 mo) and cumulatively (12-, 24-, and 36-mo time points), and assessed latency (event onset) of pertinent adverse drug reactions (ADRs) in all pts treated in PALOMA-1, -2, and -3.

Results: A total of 1352 pts were pooled for this analysis; 872 pts received PAL+ET (527 pts, PAL+LET; 345 pts, PAL+FUL). Median duration of trt was 421 days in PALOMA-1 (January 2015), 603 days in PALOMA-2 (February 2016), and 330 days in PALOMA-3 (July 2015). PAL+LET was received by 119 pts as first-line trt in PALOMA-1 and 2 for 24–<30 months and 11 pts were treated for >36 mo. PAL+FUL was received by 140 pts for >12 mo as second-line trt in PALOMA-3. The most commonly reported ADRs across all studies were neutropenia, fatigue, nausea, anemia, and leukopenia. The 6-mo-interval analyses of the most common (>15%) AEs (by preferred term [PT]) from PALOMA-1, -2, and -3 indicated that these AEs were reported with the highest frequency during the first 6-mo interval and typically decreased in incidence over time to 30–<36-mo; the most common hematologic AEs (clustered PTs) are shown (Table). The cumulative incidence of AEs after the first vs the second and third years showed similar frequencies of most AEs, including the most common ADRs.

Conclusions: Based on these long-term safety analyses, there is no evidence of specific cumulative or delayed toxicity resulting from prolonged trt with PAL+ET for HR+ HER2– ABC. This supports the ongoing investigation of PAL+ET in early breast cancer (NCT02513394).

Table. Pooled hematologic AEs: all grades and all causality clustered PTs reported for ≥10% of PAL+ET (LET/FUL)-treated pts

Time interval, mo	0–<6	6–<12	12–<18	18–<24	24–<30	30–<36	≥36
Patients, N	872	676	491	289	119	27	11
TEAEs, %							
Neutropenia	75.7	58.6	49.3	49.8	42.9	37.0	54.5
Leukopenia	40.0	27.4	16.7	11.8	7.6	11.1	18.2
Anemia	20.8	12.7	10.0	11.1	9.2	11.1	18.2
Thrombocytopenia	15.1	8.7	6.1	5.5	5.9	14.8	36.4

TEAEs=treatment-emergent adverse events.

Sponsor: Pfizer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-08

Title: A single-arm phase 2 study to assess clinical activity, efficacy and safety of enzalutamide with trastuzumab in HER2+ AR+ metastatic or locally advanced breast cancer

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Body: Background: Androgen receptor (AR) expression has been observed in up to 77% of human epidermal growth factor receptor 2-positive (HER2+) breast cancer (BC). **References:**¹ Enzalutamide (ENZA) is a potent AR inhibitor approved for patients (pts) with metastatic castration-resistant prostate cancer. In vitro, ENZA enhances antitumor activity of trastuzumab in HER2+ AR+ cell lines and inhibits proliferation in trastuzumab-resistant HER2+ cell lines.²

Methods: Pts with metastatic or locally advanced BC that was HER2+ AR+ by local or central laboratory assessment were enrolled in a single-arm, Simon 2-stage phase 2 study (NCT02091960). Key eligibility criteria included availability of a tissue sample, presence of measurable or evaluable disease per RECIST v1.1, progression on prior trastuzumab and ≥ 1 prior line of anti-HER2 therapy as the most recent regimen. Brain metastases and history of seizure were exclusionary. Evaluable pts were those with centrally confirmed nuclear AR expression $\geq 10\%$ by immunohistochemistry who received ≥ 1 dose of ENZA and had ≥ 1 postbaseline tumor assessment. Pts received ENZA 160 mg daily and trastuzumab 6 mg/kg every 21 days until disease progression. The primary objective was clinical benefit rate at 24 weeks (CBR24), defined as complete or partial response (CR or PR) or stable disease (SD) for ≥ 24 weeks in evaluable pts. Additional endpoints included safety and progression-free survival (PFS). CBR24 in ≥ 3 of 21 evaluable pts was required to continue to stage 2 and enrollment of up to 66 evaluable pts total. This design yields a 1-sided type 1 error of 5% and 90% power when the true response is 25%.

Results: Here we present results from stage 1 (data cutoff: Mar 23, 2016), with 22 evaluable pts enrolled (pts 21 and 22 enrolled simultaneously); 18 had received ≥ 4 prior lines of therapy. Median duration of ENZA exposure was 144 days (range, 22-495), mean number of complete trastuzumab infusions was 6.5. CBR24 was 27.3% (95% confidence interval [CI], 10.7-50.2); 2 confirmed PR and 4 SD ≥ 24 weeks. Median PFS was 108 days (95% CI, 56-144). All pts experienced ≥ 1 adverse event (AE) any grade; 5 pts experienced AEs grade ≥ 3 . ENZA-related AEs were reported in 16 pts (72.7%), the most common (in $\geq 10\%$ of pts) were fatigue (22.7%), nausea (18.2%), diarrhea (13.6%) and arthralgia (13.6%). Serious AEs were reported in 6 pts (27.3%); 2 each of infection and back pain, 1 each of abdominal pain, nausea, vomiting, pyrexia, urinary retention and pulmonary edema). Two pts discontinued due to drug-related AEs: 1 related to both drugs, 1 related to trastuzumab. One on-study death from pulmonary edema was reported, which was not considered related to either drug.

Conclusion: Stage 1 met its primary objective. No new safety signals were identified, and the safety profile in this study was similar to that in men with prostate cancer and women with other BC subtypes treated with ENZA. These results are encouraging for a heavily pretreated population with advanced HER2+ AR+ BC. Enrollment in stage 2 continues with the combination of ENZA and trastuzumab.

1. Micello D et al. *Virchows Arch*. 2010;457:467-476.

2. Richer J. Presented at AACR Advances in Breast Cancer, San Diego, CA, 2013.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-09

Title: A phase 2 open label study of everolimus in combination with endocrine therapy in resistant hormone receptor-positive HER2-negative advanced breast cancer

Yardley DA A, Blakely L, Hemphill B, Joseph M, Liggett W, Daniel B, Castellon A, Shastry M, Finney L, DeBusk L, Hainsworth JD D and Burris III HA A. Sarah Cannon Research Institute/Tennessee Oncology, PLLC, Nashville, TN; Sarah Cannon Research Institute/Tennessee Oncology, PLLC, Chattanooga, TN; Memorial Cancer Institute, Hollywood, FL and Sarah Cannon Research Institute, Nashville, TN.

Body: Background: Therapies targeting estrogen receptor (ER) signaling are standard for patients (pts) with hormone receptor positive (HR+) (ER and/or progesterone receptor [PR] positive) metastatic breast cancer (MBC). Dysregulation of the mammalian target of rapamycin (mTOR) pathway has been associated with endocrine therapy (ET) resistance. BOLERO-2 demonstrated that the addition of the mTOR inhibitor, everolimus (EVE), to exemestane doubled the PFS in HR+ HER2- MBC pts who previously progressed on nonsteroidal aromatase inhibitor therapy. The premise of this phase 2 trial in HR+ MBC is that the addition of EVE to the last ET on which the disease progressed may restore sensitivity to ET and extend the benefit of the anti-estrogen therapy.

Methods: Pts ≥ 18 yrs with HR+, HER2- unresectable, locally recurrent, or MBC refractory to ET in either the adjuvant or advanced/metastatic setting. 0-1 chemotherapy (chemo) regimens for MBC were permitted. Post-/ pre-/peri-menopausal women were eligible with ovarian function suppression permitted. Additional eligibility requirements include: no prior mTOR inhibitor therapy, measurable or evaluable disease, ECOG ≤ 2 , adequate bone marrow and organ function. EVE (10 mg PO daily) was administered on a 4-wk cycle in combination with the same dose and schedule of the last ET to which their disease became resistant. Disease assessments were performed every 2 cycles and treatment continued until disease progression or unacceptable toxicity. Blood samples and archival tumor were collected respectively for the VeriStrat Assay and for the Foundation One molecular profiling platform.

Results: 48 pts were enrolled; data from 26 pts is presented. Median age 63.5 yrs (range, 36-81) with 46% ≥ 65 yrs. 14 (54%) pts had received chemo in the adjuvant setting, 9 pts (35%) received chemo for MBC, and 4 pts (15%) received chemo in both settings. All pts had at least 1 prior hormonal therapy; 9 pts received ≥ 3 hormonal agents. EVE was combined with tamoxifen (27%), AIs (61%), and fulvestrant (12%). Median time on treatment was 18.6 wks (range 1-48.9 weeks). 5 pts (19%) remain on treatment and 21 (81%) have discontinued therapy due to: disease progression - 17, toxicity -2, and other causes - 2. 23 pts were evaluable for response. 1 pt on fulvestrant plus EVE had a PR and 18 pts (78%) had SD as best response, with SD > 6 mos in 7 pts, for a clinical benefit rate (CR+PR+ SD ≥ 6 months) of 35%. With a median follow up of 11 mos (range 2-16 mos), the median PFS was 6.6 months (range 3.6-9.4); the median OS has not been reached. Treatment-related adverse events consisted mostly of stomatitis, rash and fatigue with few G3 events: stomatitis 3 pts, rash 2 pts, and 1 each of fatigue, edema, and neutropenia. G1 pneumonitis was present in 2 pts. There were no G4 events or treatment related deaths.

Conclusions: In HR+ HER2- advanced/MBC patients who progressed on prior ET, the addition of EVE to the ET to which their disease became resistant, resulted in 1 PR and 7 pts with SD > 6 mos. The results of the full study population will be presented. Modulation of the mTOR/AKT/PI3K pathway with EVE may extend the benefit of ET, even after tumor progression on ET alone.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-10

Title: Evaluation of the effects of palbociclib (PAL) + letrozole (LET) on QTc

Ruiz A, Gauthier E, Durairaj C, Huang X, Hoffman J, Finn RS S, Moulder S, Joy AA A, Ettl J, Rugo HS S and Wang D. Pfizer Inc, La Jolla, CA; University of California, David Geffen School of Medicine, Los Angeles, CA; University of Texas, MD Anderson Cancer Center, Houston, TX; Cross Cancer Institute, University of Alberta, Edmonton, AB, Canada; Frauenklinik und Poliklinik Klinikum rechts der Isar, Technische Universitaet Muenchen, Muenchen, Germany and University of California San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, CA.

Body: Background: PAL, an oral cyclin dependent kinase (CDK) 4/6 inhibitor, is under investigation in multiple oncologic clinical trials and is currently approved for use in multiple countries in patients with hormone receptor positive (HR+), human epidermal growth factor receptor 2 negative (HER2-) advanced breast cancer (ABC).

International Conference on Harmonization guidance recommends that all new drugs be evaluated for effects on cardiac repolarization in a well-controlled clinical study. For drugs for which such evaluation cannot be conducted in healthy volunteers (eg, most non-adjuvant anticancer agents), collection of robust corrected QT (QTc) interval data from a dedicated QTc study (hybrid thorough QT/QTc study) in patients [pts] is required in the registration dossier. The phase 3 PALOMA-2 study (N=666) confirmed the superior clinical benefit of PAL+LET vs placebo (P) + LET in postmenopausal women with estrogen receptor positive/HER2- ABC who have not received any prior systemic anticancer therapies for ABC. One of the secondary objectives of the study was to evaluate the effects of PAL+LET on QTc.

Methods: 12 lead (with a 10 second rhythm strip) tracings were performed in triplicate ~2 min apart but within 10 min for all 3 ECGs. On the day preceding the initiation of treatment (Day 0), triplicate ECGs were obtained at time 0, 2, 4, 6, and 8 hrs (baseline). On Cycle 1 Day 14, when PAL concentrations were at steady-state, triplicate ECGs time-matched to baseline ECGs collected on Day 0 (± 35 min) were obtained following PAL or P dosing. All ECGs were sent to a central laboratory for blinded manual adjudication and these data were used for analysis. ECG measurements included PR interval, QT interval, RR interval and QRS complex. The QT interval was corrected for the effect of heart rate using Fridericia's correction (QTcF), Bazett's correction (QTcB), and a study-specific correction factor (QTcS).

Approximately 60 pts were to be included for QTc evaluation to ensure 40 evaluable pts in the PAL+LET arm (2:1 randomization) of PALOMA-2 and thus, to establish noninferiority between post-baseline and baseline (Δ QTc) at all 5 QTc sampling timepoints on Cycle 1 Day 14 with 90% power. The test was based on a 1-sided difference in means t test for paired Δ QTc ($\alpha= 0.05$). The difference in means between Δ QTc under the alternative hypothesis is 10 ms, assuming a noninferiority margin of 20 ms and the standard deviation of the paired differences equal to 16 ms based on PALOMA-1 study. If the upper bounds (UB) of 1-sided 95% confidence intervals (CI) of Δ QTc for all 5 QTc time points were < 20 ms, the post-baseline QTc will be considered noninferior to baseline and PAL+LET effect on QTc will be concluded to be not of clinical relevance.

Results: A total of 77 pts were enrolled for intensive QTc assessment in PAL+LET arm. No pts had a post-baseline absolute maximum QTcF, QTcS or QTcB ≥ 500 ms or a Δ QTc ≥ 60 ms during the intensive QTc assessment period. A random effect analysis of the mean Δ QTc data demonstrated that the UB of the 1-sided 95% CI for QTcF, QTcS, and QTcB were < 8 ms at all 5 QTc sampling time points.

Conclusion: PAL+LET does not have a clinically relevant effect on QTc.

Sponsor: Pfizer

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-11

Title: Combined targeted therapies for advanced triple negative breast cancer: A phase II trial of nab-paclitaxel and bevacizumab followed by maintenance targeted therapy with bevacizumab and erlotinib

Specht JM M, Gadi VK K, Gralow JR R, Korde LA A, Linden HM M, Salazar LG G, Rodler ET T, Cundy A, Buening BJ J, Baker KK K, Redman MW W, Kurland BF F, Garrison MA A, Smith JC C, vanHaelst C and Anderson JE E. University of Washington, Seattle, WA; UC Davis Comprehensive Cancer Center, Sacramento, CA; Seattle Cancer Care Alliance, Seattle, WA; Clinical Biostatistics, Fred Hutchinson Cancer Research Center, Seattle, WA; Biostatistics, University of Pittsburgh, Pittsburgh, PA; Confluence Health, Wenatchee, WA; SCCA EvergreenHealth, Kirkland, WA and Katmai Oncology Group, Anchorage, AK.

Body: Background: Chemotherapy remains the mainstay of therapy for patients with metastatic triple negative breast cancer (TNBC). We hypothesized that the addition of biologic agents targeting key pathways (bevacizumab targeting angiogenesis and erlotinib directed against EGFR) may prolong progression free survival (PFS) and offer a novel treatment strategy free from chemotherapy for patients with metastatic TNBC.

Methods: Patients with TNBC receiving initial therapy for metastatic disease were eligible for this multicenter phase II trial (NCT00733408) conducted at an academic center and affiliated, community practice sites. Induction therapy included nab-paclitaxel 100 mg/m² IV Qweek (wk) and Bevacizumab 10 mg/kg IV Q2wks x 24 weeks. Patients free of progression at 24 wks began maintenance therapy with bevacizumab 10 mg/kg IV Q2wks and erlotinib 150 mg po daily until progression with radiographic assessment every 8 wks. Primary objective was PFS with secondary objectives of response rate, overall survival (OS) and safety. All eligible patients were included in the analysis of PFS and OS. Response was evaluated among patients with measurable disease by RECIST 1.1 with central review. Patients with inadequate disease assessments were coded as non-responders. Kaplan-Meier method was used to estimate PFS and OS with patients censored at date of last tumor assessment (PFS) or date of last follow up (OS).

Results: From April 2009 – December 2015, 58 patients (median age 54, range 33-83) were enrolled; 56 (97%) had measurable disease, and all had metastatic TNBC by local assessment. 33 (57%) patients completed induction; 22 (38%) came off study during induction; 3 (5%) continue on maintenance therapy. 4 patients discontinued therapy prior to first assessment. As of June 8, 2016, 53 patients (91%) have progressed. Median follow up for surviving patients is 14.5 months (range 4.1-65.4). Median PFS is 7.7 months (95% CI 5.7, 9.5). Of 56 patients with measurable disease, 38 (66%) had partial response (PR); 10 (17%) with stable disease for clinical benefit rate (CBR) of 86%. Median OS is 18.2 months (95% CI 16.3, 24.5). Most common grade 3-4 toxicities during induction were neutropenia [17 (29%), 1 grade 4], fatigue [13 (22%), all grade 3], leukopenia [7 (12%), all grade 3], and neuropathy [7 (12%), all grade 3]. Rash was most common \geq grade 3 toxicity during maintenance [4 (7%), grade 3]. One patient experienced clinical CHF during maintenance month 16 requiring bevacizumab discontinuation. **Conclusions:** Nab-paclitaxel and bevacizumab followed by maintenance targeted therapy with bevacizumab and erlotinib was well tolerated. While the observed PFS did not meet pre-specified criteria of interest, the majority of patients experienced clinical benefit (86%) with 30 (57%) receiving maintenance targeted therapy. Correlative studies are ongoing. Supported by Genentech (OSI4266s), Celgene (AX-CL-BRST-PI-003828) and Janssen.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-12

Title: Ribociclib + fulvestrant in postmenopausal women with HR+, HER2– advanced breast cancer (ABC)

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Body: Background: Endocrine therapy (ET) is the treatment backbone for hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2–) ABC, but efficacy is limited by ET resistance. The cyclin-dependent kinase (CDK) 4/6–cyclin D (*CCND1*)–retinoblastoma (Rb) and phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathways have been implicated in ET resistance. CDK4/6 and PI3K/mTOR inhibitors act synergistically with ET in preclinical and clinical studies of HR+ breast cancer. Ribociclib (LEE011; CDK4/6 inhibitor) + fulvestrant ± alpelisib (BYL719) or buparlisib (BKM120) in HR+, HER2– ABC is being investigated in a Phase Ib/II study (NCT02088684). Here, we present results from the ribociclib + fulvestrant combination, with intermittent and continuous ribociclib dosing.

Methods: Postmenopausal patients (pts) with HR+, HER2– ABC refractory to aromatase inhibitors received ribociclib intermittently (600 mg/day, 3-weeks-on/1-week-off; Arm A) or continuously (400 mg/day; Arm B; following Arm A safety evaluation) + fulvestrant (500 mg; Cycle 1 Day 1 and 15; subsequent cycles Day 1). Primary objective: dose-limiting toxicities (DLTs) to confirm the recommended Phase II dose of ribociclib + fulvestrant. Secondary objectives: safety, pharmacokinetics, and preliminary antitumor activity (RECIST v1.1); biomarkers that may correlate with response were also assessed.

Results: As of March 10, 2016, 24 pts received ribociclib + fulvestrant (Arm A, n=13; Arm B, n=11); 4 pts in Arm B were ongoing; median duration of exposure was 7.4 (Arm A) and 4.5 (Arm B) months. Median number of prior regimens: 4 (Arm A) and 3 (Arm B). Treatment discontinuation (n; Arm A, Arm B) was due to disease progression (11, 4), physician decision (1, 2), and adverse events (AEs; 1, 1). DLTs in Cycle 1 (n; Arm A, Arm B) were Grade [G] 3 pulmonary embolism (1, 0) and G3 aspartate aminotransferase elevation (0, 1). The most common G3/4 drug-related AE (Arm A, Arm B) was neutropenia (62%, 36%); 5 pts had QTcF prolongation >60 ms (n; 4, 1).

Common all-Grade drug-related AEs (>35% pts) n (%)	Arm A (n=13)	Arm B (n=11)
Neutropenia	10 (77)	7 (64)
Fatigue	9 (69)	3 (27)
Nausea	6 (46)	5 (46)
Anemia	6 (46)	0 (0)
Reduced appetite	5 (39)	1 (9)

Best overall responses (BORs; n; Arm A, Arm B): partial response (PR; 3, 1), stable disease (SD; 9, 6), and neither complete response nor progressive disease (NCRNPD; non-measurable disease; 1, 4). Overall response rate: 23% (Arm A) and 9% (Arm B); disease control rate (BOR of complete response, PR, SD, or NCRNPD): 100% in both arms. Next-generation sequencing data (n; Arm A, Arm B) were available for 16 pts (7, 9): 5 pts had *CCND1* alterations (PR [1, 0], SD [2, 1], and NCRNPD [0, 1]); 11 pts had *PIK3CA* alterations (PR [1, 0], SD [3, 4], and NCRNPD [1, 2]); 2 of these pts had both *CCND1* and *PIK3CA* alterations (SD [1, 0] and NCRNPD [0, 1]).

Conclusions: Ribociclib + fulvestrant has a manageable safety profile and shows preliminary clinical activity in pretreated pts with HR+, HER2– ABC. Both ribociclib intermittent and continuous dosing schedules were well tolerated. Clinical responses were

observed in tumors with and without CDK4/6–cyclin D–Rb and PI3K/mTOR pathway alterations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-13

Title: Everolimus plus trastuzumab and paclitaxel as first-line therapy in women with HER2+ advanced breast cancer: Overall survival results from BOLERO-1

Yardley D, Hurvitz S, Jiang Z-f, Toi M, Burris H, Buyse M, Slamon D, Makhson A, Elsaid A, Lerzo G, Hellerstedt B, Nuzzo F, Sohn J, Manzyuk L, Cabaribere D, Lincy J, Weimann A, Noel-Baron F, Pacaud L and Andre F. Sarah Cannon Research Institute and Tennessee Oncology, PLLC, Nashville, TN; University of California, Los Angeles (UCLA), Los Angeles, CA; Beijing 307 Hospital of PLA, Beijing, China; Graduate School of Medicine, Kyoto University, Kyoto, Japan; Sarah Cannon Research Institute, Nashville, TN; International Drug Development Institute (IDDI), Louvain La Neuve, Belgium; University of California, Los Angeles (UCLA), Angeles, CA; Moscow Municipal Hospital No. 62, Moscow, Russian Federation; Clinical Research Centre, Faculty of Medicine, Alexandria University, Alexandria, Egypt; Sanatorio de la Providencia, Buenos aires, Argentina; Texas Oncology, Austin, TX; IRCCS Fondazione G. Pascale, Dipartimento di Senologia, Napoli, Italy; Severance Hospital, Yonsei University Health System, Seoul, Korea; Russian Cancer Research Centre, Moscow, Russian Federation; Translational Research in Oncology (TRIO), Paris, France; Novartis Pharma AG, Basel, Switzerland and Institut Gustave Roussy, Université Paris Sud, Villejuif, France.

Body: Background

Everolimus (EVE), an mTOR inhibitor has shown activity in HER2+ advanced breast cancer (ABC) in both preclinical and clinical studies. In the pivotal BOLERO-1 trial (NCT00876395), the progression-free survival (PFS) was not significantly different between the EVE + trastuzumab (TRAS) + paclitaxel (PAC) combination and placebo (PBO) + TRAS + PAC in the full HER2+ population (EVE, 15.0 mo vs PBO, 14.5 mo; HR=0.89; 95% CI: 0.73-1.08; p=0.1166). Although not reaching protocol defined level for statistical significance, the hormone receptor negative (HR-) subpopulation appeared to benefit from EVE, with a 7.2 mo PFS benefit vs PBO arm (EVE, 20.3 mo vs PBO, 13.1 mo; HR=0.66; 95% CI: 0.48-0.91; p=0.0049). The final exploratory overall survival (OS) analysis from the study is presented here.

Methods

In this phase 3 randomized trial, 719 women with HER2+ ABC without prior TRAS or chemotherapy in the metastatic setting were randomized 2:1 to receive either EVE (10 mg/d) or placebo (PBO) and weekly PAC+TRAS, stratified by visceral metastasis (lung, liver, peritoneal or pleural: yes vs no) and prior adjuvant or neo-adjuvant treatment with TRAS (yes vs no). As the primary objectives (PFS on full population and on HR- subpopulation) of BOLERO-1 were not met, the key secondary endpoint of OS was not formally statistically tested. However, given the results of PFS, in particular in the HR- subpopulation, a change to the OS analysis plan was made by introducing one final exploratory OS analysis at the time of study termination.

Results

At data cutoff (Dec 31, 2015), the median duration of exposure was 40.8 weeks (range: 0.6-320.4) in the EVE arm and 48.1 weeks (range: 1.1-308.0) in the PBO arm. After a median follow-up of 60.3 mo, 350 deaths were recorded in the full population, 238 (49.6%) in the EVE arm and 112 (46.9%) pts in the PBO arm. In the full population, the median OS was comparable in the EVE vs PBO arms (48.6 mo vs 50.0 mo respectively; HR = 1.13; 95% CI: 0.90-1.42). In the HR- subpopulation, 138 deaths were recorded; 88 (42.3%) pts in the EVE arm and 50 (48.5%) pts in the PBO arm. In the HR- subpopulation, the median OS in the EVE arm was longer compared to PBO arm (57.0 mo vs 41.6 mo respectively; HR = 0.83; 95% CI: 0.59-1.18). Stomatitis, diarrhea, alopecia, cough, rash, pyrexia, neutropenia, and fatigue were the most frequent adverse events (AEs) reported in EVE arm ($\geq 35\%$). AEs leading to dose interruption and/or change were reported in 441 (93.4%) pts in EVE arm and 165 (69.3%) pts in the PBO arm respectively. Overall, AEs leading to treatment discontinuation were reported in 262 (55.5%) pts in EVE arm and 98 (41.2%) pts in PBO arm. Serious AEs were reported in 171 (36.2%) pts in the EVE arm and 40 (16.8%) pts in the PBO arm respectively. On treatment AE related deaths were reported for 3.6% pts in the EVE arm and 0% pts in the PBO arm.

Conclusions

The median OS was similar in the EVE vs PBO arms for overall population. However, a prolongation of 15.4 mo in median OS of HR- subpopulation was observed in the EVE arm vs PBO arm in this exploratory analysis. Pts in the EVE arm had a manageable safety, consistent with the safety profile of EVE and no new safety signals were identified.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-14

Title: Single agent palbociclib with or without trastuzumab for the treatment of Rb+ advanced breast cancer

Clark AS S, Wang X, Troxel A, Burrell J, Feldman M, Lal P, Zhang P, Rosen M, Gallagher M, Ndicu J, Nivar I, Matro J, Domchek SM M, Fox KR R, O'Dwyer P and DeMichele A. Perelman School of Medicine of the University of Pennsylvania, Philadelphia, PA; Abramson Cancer Center, Philadelphia, PA; Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, PA; Perelman School of Medicine of the University of Pennsylvania, Philadelphia, PA and Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA.

Body: Background: The Cyclin D/CDK4/6/Rb axis is dysregulated in breast cancer (BC). Palbociclib (P) is an oral agent that specifically inhibits CDK 4/6 and is FDA approved for hormone-receptor positive (HR+) disease in combination with letrozole or fulvestrant. We performed a Phase II, multi-disease, basket trial of single agent P in patients (pts) with retinoblastoma positive (Rb+), advanced cancer. We now report the full expansion cohort in BC, including a subcohort of pts with HER2+ disease.

Methods: Pts with Rb+ BC were eligible for enrollment if they had measureable metastatic disease, adequate organ function and ECOG PS \geq 1, with no limit on number of prior therapies. Pts were stratified on HER2-status, with a planned HER2-positive (HER2+) cohort, with or without trastuzumab (T). Pts with active brain metastases were excluded. Pts received P 125mg for 21 days on, 7 days off of each 28 day cycle. The protocol was amended on May 12, 2014 to allow concomitant T (given every 3 weeks 6mg/kg) for pts with HER2+ disease. Toxicity was assessed every 28 days. Response was assessed every 2 cycles using RECIST 1.0 guidelines.

Results: A total of 62 pts were enrolled: 46 (74.2%) HR+/HER2-, 12% HER2+ (19.3%), and 4 (6.5%) ER/PR/HER2-. 10 of the 12 pts with HER2+ disease received T. As of June 10, 2016 one HER2+ patient (pt) remains on study who is also receiving T. Median age is 58 (range 34-88). Median prior lines of therapy is 6 (range 1-15). Response rates, progression free survival (PFS) and overall survival (OS) are depicted in Table 1 and are stratified by HER2 status and receipt of concomitant T.

Summary of Results

	Total	HR+/HER2-	HR any/HER2+ with T	HR any/HER2+ no T	HR-/HER2-
N	62*	46	10	2	4
PR	4 (6.5%)	1 (2.2%)	2 (20%)	1 (50%)	0 (0%)
SD \geq 6mo	9 (14.5%)	8 (17.4%)	1 (10%)	0 (0%)	0 (0%)
SD <6mo	22 (35.5%)	16 (34.8%)	4 (40%)	1 (50%)	1 (25%)
PD	26 (41.9%)	20 (43.5%)	3 (30%)	0 (0%)	2 (75%)
CBR (PR+SD)	13 (22%)	9 (19.6%)	3 (30%)	1 (50%)	0 (0%)
PFS (mo (95% CI))	3.5 (2.1, 5.2)	3.2 (2.0, 5.2)	6.7 (1.6, 17.8)	21.2 (5.2, 37.2)	1.7 (0.6, 4.8)
OS (mo, (95% CI))	13.5 (8.9, 14.7)	11.9 (7.8, 14.0)	17.9 (3.1, NA)	NA	7.7 (1.5, 13.5)

* 1 pt not evaluable for response. HR: hormone receptor; T: trastuzumab; PR: partial response; SD: stable disease; PD: progressive disease; CBR: clinical benefit rate; mo: months; NA: not available

The most common adverse event was Grade 3 or 4 (G3/4) neutropenia (ntp) which occurred in 30 (48%) pts. There was one episode of febrile ntp in a pt who was progressing and had received 13 prior therapies. Other G3/4 events were leucopenia (n=10, 16.1%) thrombocytopenia (n=9, 14.5%), anemia (n=3, 4.8%).

Conclusions: P is well tolerated in heavily pretreated pts. Grade 3/4 ntp occurred in 48% of pts and required dose reduction without necessity to add growth factor support; febrile ntp was uncommon. Single agent activity is modest (22% in the overall population) in this heavily pretreated cohort. 3 of the 4 partial responses observed were in pts with HER2+ disease 2 of whom received concurrent T. Prolonged stable disease was observed in 9 pts, 8 of whom had HR+ disease. Given the response rate of 30% in those with HER2+ disease receiving T, and PFS of 6.7 months, further investigation into this combination is warranted. Biomarker studies are underway.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-15

Title: Sacituzumab govitecan (IMMU-132), an anti-Trop-2-SN-38 antibody-drug conjugate (ADC) for the treatment of relapsed/refractory, metastatic triple-negative breast cancer (mTNBC): Updated results

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Body: Background. mTNBC has an aggressive course with limited effective therapy options and a median progression-free survival (PFS) of 2-4 months (mos) with standard therapy. Sacituzumab govitecan (IMMU-132) is an ADC targeting Trop-2, an antigen present in many epithelial cancers, including TNBC, and delivering SN-38, a topoisomerase I inhibitor as its therapeutic moiety. IMMU-132 was awarded *Breakthrough Therapy* designation by FDA based on its previously reported activity in relapsed/refractory mTNBC patients. Here we present updated results from the mTNBC cohort of an ongoing phase I/II study (ClinicalTrials.gov, NCT01631552).

Methods. mTNBC patients (pts) received IMMU-132 10 mg/kg on days 1 and 8 every 21 days. Trop-2 expression was not required for enrollment, but available tumor specimens underwent immunohistological (IHC) testing. Efficacy was assessed locally by RECIST 1.1; ORR, PFS and overall survival (OS) were determined for all pts. Pharmacokinetic parameters were estimated in select pts with adequate blood sampling. Immunogenicity to IMMU-132 was examined in all pts.

Results. We previously reported preliminary efficacy results in 51 mTNBC patients. Here we present data on 69 patients with data cutoff June 5, 2016. Median age was 56 years (31-81) and a median of 5 prior therapies (range 1-12), with 66 evaluable for response; ORR was 29% (19/66) 2 confirmed complete (CR) and 17 confirmed partial responses (PR). The median intention-to-treat PFS is 5.6 mos (95% CI, 3.6-7.1 mos) and median OS is 14.3 mos (95% CI, 10.5-18.8 mos). PRs included 2 pts whose tumors did not respond to anti-PD-L1 therapy. The duration of response in the 19 confirmed responders (8 continuing therapy) is 11.5 mos (95% CI = 7.6 to 12.7). The clinical benefit rate (CR+PR+SD_{≥6} mos) for the 66 assessable patients is currently 45.5%. The majority (88%) of archival tumor specimens were moderately (2+) to strongly (3+) positive by IHC for Trop-2, precluding using Trop-2 expression as a selection criterion. Among current adverse events, grade _{≥3} drug-related toxicities included neutropenia (35%), leukopenia (16%), anemia (13%), vomiting (9%), diarrhea (10%), and febrile neutropenia (4%). Clearance kinetics in 8 pts showed IMMU-132 and IgG had a terminal half-life of 15.3 ± 2.7 h and 86.5 ± 40.5 h, respectively, with area under the curve for free SN-38 (unbound) only 3% of the total amount of SN-38 (e.g., IgG bound). Thus, most SN-38 remains bound to the conjugate, and is released at a rate predicted from *in vitro* serum stability studies. No pt developed anti-IMMU-132 antibodies.

Conclusion The Trop-2-targeting ADC, IMMU-132, delivering cytotoxic doses of SN-38, shows high objective and durable tumor responses with manageable toxicity in heavily-pretreated pts with mTNBC in this updated cohort, supporting further development in this population with an unmet medical need.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-16

Title: First-line ribociclib + letrozole in patients with HR+, HER2- advanced breast cancer (ABC) presenting with visceral metastases or bone-only disease: A subgroup analysis of the MONALEESA-2 trial

Burriss HA A, Chan A, Campone M, Blackwell KL L, Winer EP P, Janni W, Verma S, Burdaeva O, Alba E, Favret AM M, Mondal S, Miller M, Germa C, Hirawat S and Yap YS. Sarah Cannon Research Institute, Nashville, TN; Breast Cancer Research Centre WA & Curtin University, Perth, Australia; Institut de Cancérologie de l'Ouest – René Gauducheau Centre de Recherche en Cancérologie, Nantes, France; Duke University Medical Center, Durham, NC; Dana-Farber Cancer Institute, Boston, MA; Universitätsklinikum Ulm, Ulm, Germany; Tom Baker Cancer Centre, Calgary, Canada; Arkhangelsk Clinical Oncology Dispensary, Arkhangelsk, Russian Federation; Hospital Universitario Virgen de la Victoria, IBIMA, Málaga, Spain; Virginia Cancer Specialists, Arlington, VA; Novartis Pharmaceuticals Corporation, East Hanover, NJ and National Cancer Center Singapore, Singapore.

Body: Background: Patients with ABC who present with visceral metastases have a worse outcome than patients with non-visceral disease, while patients with bone-only disease tend to have a better prognosis. Ribociclib (LEE011) is an oral, selective inhibitor of cyclin-dependent kinase (CDK) 4/6. In a Phase 3, placebo-controlled, randomized trial (MONALEESA-2; NCT01958021), first-line ribociclib (R) + letrozole (L) significantly prolonged progression-free survival (PFS) vs placebo (P) + L in patients with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) ABC, with a hazard ratio of 0.556 (95% confidence interval [CI]: 0.429–0.720; p=0.00000329) at the interim analysis cut-off date (Jan 29, 2016). Here, we present subgroup analyses in patients with visceral metastases, and those with bone-only disease.

Methods: Postmenopausal women with HR+, HER2- ABC were randomized 1:1 to receive R (600 mg/day; 3-weeks-on/1-week-off) + L (2.5 mg/day; continuous) or P+L, stratified by the presence of liver and/or lung metastases. No prior CDK4/6 inhibitors or systemic therapy for ABC were allowed. Eligible patients had Eastern Cooperative Oncology Group performance status ≤ 1 , baseline alanine/aspartate aminotransferase levels $< 5 \times$ upper limit of normal (ULN) or $< 2.5 \times$ ULN for patients with or without liver metastases, respectively, and ≥ 1 predominantly lytic bone lesion at baseline for patients with bone-only disease. Locally assessed PFS was analyzed for all patients (primary endpoint), and for predefined patient subgroups.

Results: Overall, 668 patients were randomized; 393 had visceral metastases and 147 had bone-only disease.

	Visceral metastases n=393		Bone-only disease n=147	
	R+L n=197	P+L n=196	R+L n=69	P+L n=78
Median age, years (range)	63 (23–91)	63 (29–88)	65 (37–85)	63 (37–84)
De novo metastatic disease, n (%)	53 (27)	55 (28)	28 (41)	24 (31)
Non-de novo disease-free interval, n (%)				
≤ 24 months	12 (6)	15 (8)	5 (7)	6 (8)
> 24 months	132 (67)	126 (64)	36 (52)	48 (62)
Discontinued treatment, n (%)	83 (42)	111 (57)	29 (42)	40 (51)
Reason for discontinuation, n (%)				
Disease progression	56 (28)	93 (47)	17 (25)	31 (40)
Patient/physician decision	10 (5)	15 (8)	7 (10)	6 (8)
Adverse events	16 (8)	3 (2)	4 (6)	2 (3)
Protocol deviation	0	0	1 (1)	1 (1)
Death	1 (<1)	0	0	0

In patients with visceral metastases: Median PFS was not reached in the R+L arm (95% CI: 19.3–not estimable [NE]) vs 13.0 months (95% CI: 12.6 –16.5) in the P+L arm, with hazard ratio 0.535 (95% CI: 0.385–0.742). Median duration of exposure was 12.0 and 13.0 months (R and L, respectively) in the R+L arm, and 12.1 and 12.2 months (P and L, respectively) in the P+L arm.

In patients with bone-only disease: Median PFS was not reached in the R+L arm (95% CI: NE–NE) vs 15.3 months (95% CI: 13.8–NE) in the P+L arm, with hazard ratio 0.690 (95% CI: 0.381–1.249). Median duration of exposure was 12.1 and 12.6 months (R and L, respectively) in the R+L arm, and 12.7 and 12.9 months (P and L, respectively) in the P+L arm.

Conclusions: First-line R+L was well tolerated and significantly prolonged PFS vs P+L in postmenopausal women with HR+, HER2– ABC, both in patients with visceral metastases and those with bone-only disease.

Keywords: Advanced breast cancer; CDK4/6 inhibitor; Letrozole; Ribociclib

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-17

Title: Safety of the combination of everolimus plus exemestane in the Italian cohort of patients enrolled in the expanded access “BALLET” study

Generali D, Bordonaro R, Febbraro A, Madoffa A, Romito S, Michelotti A, Savastano C, Mariani G, Tondini C, Piovano P, Iona MT, Bighin C, Roviello G, Ascione G, Goffredo F, Sartori D, Frassoldati A and Simoncini E. University of Trieste.

Body: Background: The expanded access “BALLET” study has been designed to evaluate the safety of EVE plus EXE combination in hormone receptor-positive (HR+), human epidermal growth factor-receptor-2-negative (HER2-) metastatic Breast Cancer (mBC). The Italian population was predominantly enrolled in trial.

Patients and methods: Patients has been included according to the inclusion and exclusion criteria provided previously in the BALLET study. The aim of our analysis was the safety everolimus and exemestane analysed in two sets of population: a subpopulation including only patients who never received chemotherapy in metastatic setting (416 patients – 36.1% of the safety population) and a subpopulation including only patients who received at least one chemotherapy in metastatic setting, whatever the line of treatment (735 patients – 63.9%).

Results: One thousand two hundred seventy nine (1279) Italian female patients were screened, 1153 (90.1% of the screened set) out of these were included in the analysis and 1151 (90.0% of the screened set) were included in the safety population. 1116 (97.0% of the safety population) prematurely discontinued the study drug and the main reasons reported were disease progression (39.1%), local reimbursement of everolimus (31.1%) and adverse event(s) (16.1%). The mean duration of study treatment exposure was 158.3 ± 106.79 days (median 139.5) for exemestane and 153.9 ± 108.48 days (median 135.0) for everolimus with a treatment compliance (higher than 90%) of 94.4% and 58.6% and (lower than 60%) of 0.1% and 15.1% for exemestane and everolimus, respectively. 92.5% of patients of the safety population (91.1% and . 93.3% patients without and with chemotherapy respectively) experienced at least one adverse event: gastrointestinal disorders” (67.3% vs. 64.6% in without and with chemo group); general disorders (48.6% vs. 48.3%); metabolism and nutrition disorders (35.6% vs. 37.4%) and skin and subcutaneous tissue disorders (32.2% vs. 27.5%). The incidence of everolimus related adverse events was higher (83.9%) when compared to those which occurred with exemestane. The most commonly reported adverse event was stomatitis (51.3% of patients) with 22.5% Grade 1; 18.2% Grade 2; 10.5% Grade 3; 0.2% Grade 4. The 49.7% of the patients experienced at least one stomatitis related to everolimus. No relevant difference was observed between the two groups of patients without and with chemo in metastatic setting.

Conclusions: The administration of chemotherapy before starting EVE plus EXE combination did not affect the safety profile of EXE/EVE in the treatment of mBC. The stomatitis is the most frequent and relevant adverse event to be clinically focused on.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-18

Title: Phase Ib safety, efficacy, and molecular analysis of ribociclib (LEE011) plus letrozole for the treatment of ER+, HER2– advanced breast cancer

Munster P, Ismail-Khan R, Garcia-Estevez L, Mayer IA A, Becerra C, Hamilton E, De Boer R, Wardley AM M, Im S-A, Teixeira L, Wang Y, Su F, Germa C, Hirawat S and Juric D. University of California San Francisco, San Francisco, CA; H. Lee Moffitt Cancer Center, Tampa, FL; Centro Integral Oncológico Clara Campal, Madrid, Spain; Vanderbilt-Ingram Cancer Center, Nashville, TN; Texas Oncology-Baylor University Medical Center-US Oncology Research, Dallas, TX; Sarah Cannon Research Institute and Tennessee Oncology, Nashville, TN; The Royal Melbourne Hospital, Melbourne, Australia; The Christie NIHR/CRUK Clinical Research Facility, Manchester, United Kingdom; Seoul National University College of Medicine, Seoul, Korea; Hopital Saint Louis and University Paris Diderot, Sorbonne Paris City, Paris, France; Novartis Pharma AG, Basel, Switzerland; Novartis Pharmaceuticals Corporation, East Hanover, NJ and Massachusetts General Hospital Cancer Center, Boston, MA.

Body: Background: Aromatase inhibitors are the standard first-line treatment for patients (pts) with estrogen receptor-positive (ER+), human epidermal growth factor receptor 2-negative (HER2–) advanced breast cancer (aBC). However, resistance via disruption of the cyclin D–cyclin-dependent kinase (CDK)4/6–inhibitor of CDK4–retinoblastoma pathway is common. The oral, selective CDK4/6 inhibitor ribociclib has demonstrated synergistic activity with letrozole in preclinical models of ER+ breast cancer. This Phase Ib study investigated ribociclib plus letrozole in ER+, HER2– aBC (NCT01872260).

Methods: Postmenopausal pts with ER+, HER2– aBC received ribociclib (600 mg/day; 3-weeks-on/1-week-off) plus letrozole (2.5 mg; continuous). The primary objective of the expansion phase was to evaluate safety and tolerability. Secondary and exploratory objectives included efficacy, pharmacokinetics (PK), and biomarkers.

Results: As of October 30, 2015, 47 pts received ribociclib plus letrozole: 19 pts in the initial enrollment and 28 pts in the expansion phase. In the initial enrollment, 17/19 pts (89%) were previously treated for aBC (PT group); in the expansion phase, 27/28 pts (96%) were treatment-naïve for aBC (first-line group). All 19 PT pts and 10 first-line pts (62% of all pts) have discontinued treatment. Common (>5% of pts) treatment-related Grade 3/4 adverse events included neutropenia (43%), neutrophil count decreased (11%), and white blood cell count decreased (6%). Complete response (CR) was observed in 1 pt (4%) in the first-line group. In the PT and first-line groups, 1 pt (5%) and 10 pts (36%) had a partial response (PR), 7 pts (37%) and 4 pts (14%) had neither CR nor progressive disease (PD), 7 pts (37%) and 10 pts (36%) had stable disease (SD), and 4 pts (21%) and 3 pts (11%) had PD, respectively. Among pts with measurable disease, overall response rate (ORR; CR + PR) was 9% (95% confidence interval [CI]: 0–41%) for 11 PT pts and 46% (95% CI: 26–67%) for 24 first-line pts, and clinical benefit rate (CBR; CR + PR + SD \geq 24 weeks) was 18% (95% CI: 2–52%) for 11 PT pts and 79% (95% CI: 58–93%) for 24 first-line pts. Ribociclib PK data were consistent with single-agent data and indicated no drug–drug interaction with letrozole. Notable genetic alterations detected by next-generation sequencing included *PIK3CA* mutations (4/10 PT pts [40%]; 14/22 first-line pts [64%]) and *ESR1* mutations (2/10 PT pts [20%]). Responses were observed in pts harboring *PIK3CA* mutations (1 CR [first-line pt], 1 unconfirmed CR [first-line pt], 3 PR [1 PT pt, 2 first-line pts], and 3 unconfirmed PR [first-line pts]). Progression-free survival data for ribociclib plus letrozole, in addition to updated ORR and CBR, will be presented at the symposium.

Conclusions: The combination of ribociclib plus letrozole demonstrated encouraging clinical activity, particularly in pts treatment-naïve for aBC, with an acceptable safety profile that is in line with CDK4/6 inhibition. These results, which are consistent with the positive results of the MONALEESA-2 study (NCT01958021), provide additional safety and efficacy evidence for the use of ribociclib plus letrozole for the first-line treatment of postmenopausal aBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-19

Title: Time on treatment of everolimus versus endocrine monotherapy or chemotherapy for early-line treatment of HR+/HER2- metastatic breast cancer: A retrospective chart review study in the US

Li N, Ohashi E, Koo V, Xie J, Hao Y and Tang DH H. Analysis Group, Inc., Boston, MA; Analysis Group, Inc., Los Angeles, CA and Novartis Pharmaceuticals Corporation, East Hanover, NJ.

Body: Background:

Among postmenopausal women with hormone receptor-positive, human epidermal growth factor receptor-2-negative (HR+/HER2-) metastatic breast cancer (mBC) whose disease progressed on a non-steroidal aromatase inhibitor (NSAI), everolimus-based therapy (EVE), different endocrine monotherapies (ET mono), and chemotherapies (CT) are commonly used. Time on treatment is an outcome primarily determined by a therapy's combined efficacy and safety profile. This study assessed the real-world time on treatment (TOT) among patients receiving these treatments in early-line (i.e., 1st and 2nd) settings.

Methods:

A nationwide sample of postmenopausal HR+/HER2- mBC patients treated by community oncologists in the US was included in this retrospective chart review. Eligible patients for this study were required to fail NSAI and then receive EVE, ET mono or CT (index therapy) as an early-line therapy for mBC between July 1, 2012 and April 15, 2013. TOT was measured from index therapy initiation to physician-reported treatment discontinuation and compared among treatment groups using Kaplan-Meier analyses with log-rank tests and Cox proportional hazards models adjusting for the line of therapy and baseline characteristics including recurrent or de novo disease status, age, race, insurance type, Charlson comorbidity index, sites of metastases (e.g., bone, any other visceral site), ECOG performance status, previous CT treatment in the mBC setting, and duration from the initiation of the last adjuvant ET to mBC diagnosis.

Results:

A total of 145 patients treated with EVE, 217 patients treated with ET mono, and 102 patients treated with CT were included in the analysis. Baseline characteristics among the three treatment groups were similar, although EVE-treated patients had higher burden of metastases relative to ET mono-treated patients, but lower burden relative to CT-treated patients. TOT was longer among EVE-treated patients than ET mono- and CT- treated patients (log-rank tests: $p=0.01$ and $p<0.01$). For patients who completed their index treatment, the median TOT among EVE, ET mono, and CT treatment groups were 8.9, 5.7, and 6.1 months, respectively. After adjusting for baseline characteristics, EVE was associated with significantly longer TOT compared with ET mono [hazard ratio (HR) = 0.62, 95% confidence interval (CI): 0.45 – 0.85] and with CT (HR = 0.32, 95% CI: 0.22 – 0.46).

Conclusions:

This real-world US chart review study of postmenopausal women with HR+/HER2- mBC showed that patients receiving EVE in line 1 or 2 experienced significantly longer TOT than those receiving ET mono or CT.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-20

Title: Efficacy and safety of everolimus plus exemestane in HR+, HER2– advanced breast cancer progressing on/after prior endocrine therapy, in routine clinical practice: 2nd interim analysis from STEPAUT

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Body: Background

STEPAUT, an Austrian non-interventional study evaluated the safety and efficacy of everolimus (EVE) + exemestane (EXE) in patients (pts) with hormone receptor-positive (HR+), human epidermal growth factor receptor-2 negative (HER2–) advanced breast cancer (ABC) recurring/progressing on/after prior nonsteroidal aromatase inhibitors (NSAIs) in routine clinical practice. Results of the 1st interim analysis (IA) were consistent with BOLERO-2 data. Here we present results from the 2nd pre-planned IA.

Materials and Methods

STEPAUT, with a planned enrolment of 300 pts, included postmenopausal pts, aged ≥ 18 yrs with HR+, HER2– ABC treated with EVE+EXE, progressing on/after NSAIs. Primary endpoint was progression-free survival (PFS); secondary endpoints included response per RECIST v1.1 and safety.

Results

The 2nd IA included 225 pts with a median age of 65 yrs. At the time of data cut-off: 9 May, 2016, 147 pts had discontinued study treatment, mainly due to disease progression and adverse events (AEs). Median duration of follow-up was 6.5 months (range 0–26.3 months), 172 pts (95%) had ECOG PS 0-1 and 52% of pts had visceral metastasis. A majority of pts (n=109, 54%) received the approved EVE dose of 10mg as the start dose, while 91 pts (45%) received half of the approved EVE dose i.e. 5mg. Median PFS values for different subgroups are shown in table 1 below. Overall, 57 pts (28%) required therapy interruption while 37 pts (18%) had EVE dose reduction from 10 to 5mg. A decreasing trend in AE frequency irrespective of EVE dose was observed during treatment period. The majority of AEs were of mild to moderate severity; most frequent AEs (all grades) were stomatitis, mucositis (48.0%), exanthema, rash (22.2%), and dyspnea, cough (22.2%). Frequent grade 3 or 4 AEs were stomatitis, mucositis (4.4%), weight loss, reduced general condition (2.7%), and inappetence, nausea (2.2%). Median time to first occurrence of stomatitis was 0.5 months; 8 pts (5%) discontinued therapy due to stomatitis and/or rash. Serious AEs constituted 10% of all AEs.

Table 1: Median PFS across various subgroups

	PFS (months)	95% Confidence interval
Overall (n=206)	9.5	7.7-10.7
Dose		
EVE 10mg (n=68)	9.1	6.4-10.7
EVE 5mg (n=53)	6.5	3.7-12.5
EVE 5 to 10mg (n=17)	11.3	5.4-15.4
EVE 10 to 5mg (n=27)	9.5	7.7-14.7
Therapy lines		
First* (n=12)	11.3 [#]	8.4-14.2
Second (n=24)	11.3	11.3-11.3
Third (n=25)	13.3	6.5-23.7

Fourth (n=14)	10.0	5.5-10.0
Fifth and beyond (n=11)	7.3	5.6-8.7
Stomatitis and/or rash incidence		
Stomatitis (n=73)	9.1	5.5-11.9
Rash (n=16)	9.2	3.4-18.4
Stomatitis and rash (n=34)	11.5	10.3-19.0

*only 2 events, #mean value

Conclusions

Real world data from STEPAUT support EVE+EXE as a suitable treatment option for HR+, HER2- ABC recurring or progressing on/after prior NSAIs. Overall safety profile was also consistent with previous reports. Of note, occurrence of stomatitis and/or rash did not negatively influence PFS. Furthermore, a lower start dose of EVE 5mg did not seem to affect PFS negatively as long as the dose was adjusted to 10mg after a short period of time, thus supporting the administration of the approved EVE 10mg/day dose in the routine clinical setting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-21

Title: NCI9782: A phase 1 study of talazoparib in combination with carboplatin and paclitaxel in patients with advanced solid tumors

Turk A, Chan N, Leal T, O'Regan R, Tevaarwerk A, Rice L, Campbell T, Barroilhet L, Mehnert J, Eickhoff J, Kolesar J, Liu G and Wisinski K. University of Wisconsin Carbone Cancer Center, Madison, WI and Rutgers Cancer Institute of New Jersey, New Brunswick, NJ.

Body: *Background:* Poly(ADP-ribose) polymerase (PARP) enzymes are involved in DNA repair and activated by DNA strand breaks. DNA damage from carboplatin is associated with activation of PARP. Preclinical data indicate that PARP inhibition potentiates the anti-tumor effect of platinum chemotherapy. Talazoparib (T) is an oral, selective PARP inhibitor with a single agent maximum tolerated dose (MTD) of 1mg orally qd. Primary dose-limiting toxicity (DLT) was thrombocytopenia. This phase I study combines T with the commonly used chemotherapy regimen of carboplatin (C) and paclitaxel (P).

Methods: Two dosing schedules are being investigated. In both schedules, C is administered on day 1 and P on days 1, 8, and 15 of a 21-day cycle. T (100-1000mcg) is dosed once daily for days 1-7 (schedule A) or days 1-3 (schedule B) starting on day 1. A 3+3 design is used for dose escalation. Key eligibility criteria include age ≥ 18 with a measurable or evaluable solid incurable malignancy. Patients (pts) must have tumor type that is expected to respond to C + P or have BRCA germline or somatic mutation. Stable, treated brain metastases are allowed. No prior C for metastatic disease is allowed. Pts must have platelets >150 and no need for anticoagulation. After 4-6 cycles of combination therapy, pts may continue the combination, change to C and intermittent T without P or change to T alone with continuous daily dosing. Each schedule will have a 6 subject dose expansion at the MTD. The starting dose level for schedule B will be the MTD from schedule A.

Results: Schedule A cohort results are reported: 11 pts (median age 59 [range 39-68]; 8 female; 3 male) have been enrolled. Pts had breast (6), ovarian (2), pancreatic (1), and SCC of oropharynx (1) and concurrent ovarian and pancreatic (1). Five pts are BRCA2+ and 3 pts are BRCA1+. Dose level 3 on schedule A (T 350mcg with C AUC 6 and P 80mg/m²) exceeded the MTD with 2 of 3 pts experiencing hematologic dose limiting toxicities (DLTs) including 1 pt with grade (gr) 3 neutropenic fever and gr 4 thrombocytopenia and another pt with grade 3/4 neutropenia lasting > 7 days. Most common AEs include neutropenia (grade 3-4: 7), anemia (grade 3-4: 3), and thrombocytopenia (grade 3-4: 4). Results from expansion of dose level 2 (T 250mcg with C AUC 6 and T 80mg/m²) will be presented. The 11 pts were on study a median of 9 weeks (range 9-36+). Four pts have discontinued study therapy: 1 due to need for anticoagulation for PE, 1 for prolonged cytopenias, and 2 for disease progression. Of the 8 pts with measurable disease evaluated for response to date, 4 had SD, 1 had a cPR, 1 had radiographic CR, and 2 with PD. A pt with BRCA 1+ triple negative breast cancer has maintained a prolonged PR (36+ weeks) even after dose reductions to T 100mcg with C AUC 3. One pt with ovarian cancer (BRCA WT) has radiographic CR (CA 125 remains mildly elevated) after 15+ weeks of therapy.

Conclusion: PARP inhibition with talazoparib days 1-7 in combination with carboplatin and paclitaxel leads to DLT of myelosuppression. However, clinical responses are seen even with lower dose combinations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-22

Title: Cobimetinib (C) combined with paclitaxel (P) as a first-line treatment in patients (pts) with advanced triple-negative breast cancer (COLET study): Updated clinical and biomarker results

Brufsky A, Kim S-B, Velu T, García-Saenz JA A, Tan-Chiu E, Sohn JH, Dirix L, Borms MV V, Liu M-C, Moezi MM M, Kozloff MF F, Sparano JA A, Xu N, Wongchenko M, Simmons B, McNally V and Miles D. University of Pittsburgh, Pittsburgh, PA; Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; Chirec Cancer Institute, Brussels, Belgium; Hospital Clinico San Carlos, Madrid, Spain; Florida Cancer Research Institute, Plantation, FL; Severance Hospital, Yonsei University Health System, Seoul, Korea; Sint-Augustinuskliniek, Antwerp, Belgium; AZ Groeninge, Kortrijk, Belgium; Koo Foundation Sun Yat-Sen Cancer Center, Taipei, Taiwan; Cancer Specialists of North Florida, Jacksonville, FL; Ingalls Memorial Hospital, Harvey, IL; Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; Genentech, Inc., South San Francisco, CA; Roche Products Ltd., Welwyn Garden City, United Kingdom and Mount Vernon Cancer Centre, London, United Kingdom.

Body: Resistance to standard taxane-based chemotherapy is common in triple-negative breast cancer (TNBC). Mutations and gene amplifications in the MAPK pathway that upregulate MAPK signaling are present in many TNBC tumors. Upregulation of the MAPK signaling pathway can result in degradation of the pro-apoptotic protein BIM and upregulation of anti-apoptotic proteins, including BCL-2, BCL-XL, and MCL-1, thus promoting cell survival and desensitizing tumor cells to the pro-apoptotic effects of taxane chemotherapy. Updated data on clinical safety and efficacy are presented along with biomarker data evaluating the effects of treatment on induction of apoptosis. The COLET study (ClinicalTrials.gov ID, NCT02322814; EudraCT number, 2014-002230-32) consisted of a safety run-in (n~12) followed by a blinded 1:1 randomized expansion stage (n~90) to C + P or placebo (PBO) + P. The safety stage is complete and the randomized stage is enrolling pts. Two additional cohorts investigating the effect of adding atezolizumab will be recruiting and are out of scope of this submission. Pts in cohort I were treated with P 80 mg/m² on days 1, 8, and 15 and C/PBO 60 mg/day on days 3–23 of each 28-day cycle until disease progression or unacceptable toxicity. Gene expression and apoptotic index were measured by RNA-Seq and TUNEL staining, respectively, to assess the biologic activity of C + P. Sixteen women (median age, 55.5 years) were enrolled in the safety run-in stage. At data snapshot (April 22, 2016), all 16 pts had received ≥1 dose of study treatment. Median time on treatment was 116 days (range, 7-336) for C and 84 days (range, 0-351) for P. Fifteen (94%) pts had ≥1 adverse event (AE); 5 (31%) pts had grade 1/2 AEs and 10 (63%) pts had grade 3 AEs (**Table**). No pts experienced grade 4–5 AEs. Among the 16 safety run-in patients, responses to date include partial response (PR; n = 8 [50.0%]), stable disease (SD, n = 4 [25.0%]), and progressive disease (n = 2 [12.5%]), as well as 2 pts with no post-baseline tumor assessment. Six pts maintained a PR at ~20 weeks and three maintained a PR at ≥40 weeks. To date, matched pre- and on-treatment biopsies were evaluable for 2 pts, 1 with a PR and 1 with SD. In the patient who attained a PR, increased expression of pro-apoptosis genes, including BIM, was observed; but this was not seen in the patient experiencing SD. The PR patient also had an increase in apoptotic index. Updated biomarker data will be reported. This is the first study to evaluate C + P in TNBC. The safety profile of C + P is consistent with that of known safety profiles. Efficacy and safety will be further evaluated in the ongoing randomized stage.

Most common (any grade ≥20%) AEs

Treatment-emergent AEs, n (%)	C + P (safety run-in stage), N = 16	
	All grades[bold]	Grade 3
Diarrhea	10 (63)	1 (6)
Rash	8 (50)	0
Nausea	7 (44)	0
Alopecia	5 (31)	0
Blood CPK level increase	5 (31)	1 (6)
Stomatitis	4 (25)	2 (13)

Asthenia	4 (25)	1 (6)
Constipation	4 (25)	0
Dyspnea	4 (25)	0
Edema peripheral	4 (25)	0
Pyrexia	4 (25)	0
Vomiting	4 (25)	0

AEs, adverse events; C, cobimetinib; CPK, creatinine phosphokinase; P, paclitaxel.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-23

Title: Phase II trial of selinexor for metastatic triple negative breast cancer

Han HS S, Ismail-Khan R, Carney D, Extermann M, Hogue D, Soliman H, Loftus L, Lee JK K and Sullivan D. H.Lee Moffitt Cancer Center and Research Institute, Tampa, FL.

Body: Background: XPO1 (also known as CRM1) is the exclusive nuclear exporter of multiple tumor suppressor proteins (TSP) including p53, p21, BRCA1/2, pRB, FOXO. XPO1 inhibition forces nuclear accumulation of TSPs, thus inducing apoptosis in cancer cells. Selective Inhibitors of Nuclear Export (SINE) compounds are novel, small molecule, slowly reversible inhibitors of XPO1. SINE compounds showed potent cytotoxicity in the majority of TNBC cell lines in vitro and xenografts.

Methods: Eligible patients had measurable metastatic TNBC, received at least one prior line of chemotherapy in the setting of metastatic disease (including anthracycline and taxane), and had an ECOG PS 0-1. Selinexor was given at 60 mg orally twice weekly (day 1 & 3 of each week), three of each four-week cycle. The primary objective of this study was to determine the clinical benefit rate (CBR, defined as complete response + partial response + stable disease (SD) ≥ 12 weeks) from Selinexor in patients with TNBC. This study used a Simon two-stage design (null hypothesis CBR 5%, alternate hypothesis CBR 20%)

Results: Ten patients with a median age of 60 years (range 44-71) were enrolled between 7/2015 and 1/2016. The median number of prior chemotherapy lines was 2 (range 1-5). A planned interim analysis for the first stage per protocol was performed. Three patients had SD and 7 had progressive disease. The median duration of SD was 84 days (range 17-130 days) and all patients experienced progressive disease with a median PFS of 38 days (range 17-130 days). Based on these results, the error probability of observing no response among the first 10 patients is 0.107 under the alternative hypothesis, i.e., P of response =20%, using the Exact binomial test. This false negative error rate is under the planned type II error 0.20, which is consistent with the predefined stopping rule of the study: the study would be halted if no patient achieved objective response (complete or partial response) in the first 10 eligible patients (stage I).

The most frequent grade 1 and 2 observed adverse events that were possibly related to Selinexor were nausea 30% (all grade 1), vomiting 50% (40% grade 1), constipation 50% (40% grade 1, 10% grade 2), diarrhea 20%, anorexia 30%, thrombocytopenia 30%, blurred vision 30%. No grade 4 toxicity was noted. Grade 3 toxicity included reversible encephalopathy (10%), irritability (10%), and thrombocytopenia (10%).

Conclusion: Selinexor was well tolerated in patients with advanced TNBC but did not result in objective responses in this patient population. Further combination studies can be explored.

2016 San Antonio Breast Cancer Symposium

Publication Number: P4-22-24

Title: A retrospective study about re-biopsy at disease progression on T-DM1

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Body: Background:

T-DM1 is an antibody-drug conjugate, which consists of trastuzumab, DM1, and linker. It has been established as standard of care for human epidermal receptor (HER) 2-positive metastatic breast cancer (MBC). At the first step, trastuzumab has to combine with extracellular domain of HER2. Then T-DM1 is delivered into HER2-positive cancer cells, and DM1 is released. After that, antitumor activity of DM1 can be exerted. In this way, T-DM1 works on HER2-positive cancer cells with high specificity. This retrospective study is aimed to examine whether HER2 status at disease progression on T-DM1 is related to efficacy of T-DM1.

Patients:

We identified 23 patients who started treatment with T-DM1 between February 2014 and March 2016 at our department. The data cut-off date was April 30, 2016.

Methods:

We retrospectively reviewed the medical records of these 23 patients and investigated the following items: patient characteristics, time to failure (TTF), objective response, and results of re-biopsy. TTF were analyzed by the Kaplan–Meier method. The definition of HER2-positive was immunohistochemistry 3+, or FISH >2.2.

Results:

There were 23 patients who started administration of T-DM1 for MBC at our department in this period. All patients had HER2-positive MBC which was diagnosed as HER2 positive by examination of samples from primary tumor. At the time of data cut-off, five patients had continued T-DM1 therapy, and 18 patients had finished it due to disease progression in 15 patients and adverse events in three patients. Re-biopsy was conducted to 13 (87%) of 15 patients who experienced disease progression during TDM-1 therapy. Samples of re-biopsy were acquired from the site that showed progression during T-DM-1 therapy. Re-biopsy revealed that HER2 status had changed to negative in six (46%) patients (Negative group), and had remained positive in seven (54%) patients (Positive group). Patient characteristics are shown in table.

Patient characteristics

	All patients (23)	Negative group (6)	Positive group (7)
Age at start T-DM1 (year) Median (rang)	61 (38-80)	63 (48-71)	58 (38-78)
ECOG PS			
0-1	21 (91%)	6 (100%)	7 (100%)
2-3	2 (9%)	-	-
ER and/or PgR positive	13 (57%)	5 (83%)	4 (57%)
ER and PgR negative	10 (43%)	1 (17%)	3 (43%)
Number of prior chemotherapy regimens for MBC Median (rang)	2 (1-9)	2 (1-9)	2 (1-5)
Prior Lapatinib	10 (43%)	2 (33%)	4 (57%)

Median TTF was 65.5 days (95% CI: 34-NA) in Negative group, 126 days (95%CI: 70-140) in Positive group, and 196 days (95%CI: 93-594) in all patients. Proportion of progressive disease (PD) was 83% (five in six patients) in Negative group, 43% (three in seven patients) in Positive group, and 39% (nine of 23 patients) in all patients.

Conclusions:

In 46% of patients who received re-biopsy, HER2 status changed to negative. There was a trend that TTF was shorter and PD rate was higher in Negative group than in Positive group. We demonstrated that some of patients who experienced PD during

T-DM1 therapy had HER2-negative MBC, and in these patients efficacy of T-DM1 was likely to be insufficient. In such a case that disease progression occurs in a few months of T-DM1 therapy, we have to consider whether its HER2 status remains positive or changes to negative.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD7-01

Title: Can surrogate pathological subtyping replace molecular subtyping? Outcome results from the MINDACT trial

Cardoso F, Slaets L, de Snoo F, Bogaerts J, van 't Veer LJ J, Rutgers EJ J, Piccart-Gebhart MJ J, Stork-Sloots L, Russo L, Dell'Orto P and Viale G. Breast Unit, Champalimaud Cancer Center, Lisbon, Portugal; European Organization for Research and Treatment of Cancer, Brussels, Belgium; Medical Affairs, Agendia, Amsterdam, Netherlands; Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco; Netherlands Cancer Institute, Amsterdam, Netherlands; Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium and European Institute of Oncology (IEO) and University of Milan, Milan, Italy.

Body: Background

Molecular subgroups within early breast cancer (EBC), such as Luminal A, Luminal B, HER-2+, Basal-like may help to best to identify patients for specific treatment regimens. Controversy exists as to which methodology is best at identifying these molecular subgroups. Immunohistochemistry (IHC) may be used as a surrogate method to stratify patients. Molecular subtyping gene expression based tests, such as BluePrint, measure a greater number of genes than pathological criteria. ER, PgR, HER-2 and Ki67 are measured individually at the protein level, while BluePrint is designed to capture the functional underlying biologic pathway regulated by these receptors.

Methods

The MINDACT trial is an international, prospective, randomized, phase III trial which has proven the clinical utility of MammaPrint in selecting EBC patients who can safely avoid chemotherapy. Here we present the results of a preplanned MINDACT sub-study to compare outcome based on molecular subtyping (MS) to surrogate pathological subtyping (PS) as endorsed by 2013 St. Gallen Consensus. MS data were obtained by MammaPrint (MP) and BluePrint classifying patients in the following subtypes: Luminal A (MP Low Risk); Luminal B (MP High Risk); HER2-type; and Basal-type. ER, PgR, HER2 and Ki67 protein status were centrally assessed by IHC/FISH. The primary hypothesis was that among PS Luminal patients, patients with HER-2+ or Basal-type tumors by MS would have a decreased DMFS compared to MS Luminal patients. At $\alpha=5\%$ with 220 events, the study has 80% power to demonstrate this for HR=2.44.

Results

The table depicts classification of tumors according to PS versus MS for all patients (n=5,806).

PS versus MS

	MS				
PS	Lum A	Lum B	HER-2+	Basal	Total
Lum A	2456	270	8	13	2747
Lum B	1069	794	22	86	1971
HER-2 enriched	118	95	318	26	557
TN	14	10	7	500	531
Total	3657	1169	355	625	5806

Most pronounced differences: MS classified 54% as Luminal A among the Luminal B by PS. MS classified 38% as Luminal (A and B) and 5% as Basal-type among the HER-2+ by PS. MS classified 5% as Luminal (A and B) among the TN cases by PS.

MS identifies 63% of patients as Luminal A, while PS identifies 47%; 5yr DMFS for both methods was $\geq 96.0\%$.

PS Luminal cancers that were classified as HER-2+ or Basal-type by MS had a lower 5yr DMFS (88.0% for HER-2+ and 90.2% for Basal), albeit non-significant, than those who were also Luminal by MS (95.9%): HR= 1.40, 95% CI = 0.75-2.60.

In PS TN cancers, MS identified 24 out of 500 patients (5%) as Luminal-type with excellent prognosis (5yr DMFS of 100% versus 71.4% for MS HER-2+ or 90.1% for MS Basal-type).

Among the PS Luminal patients, Ki67 cut at 20% identified patients with ki67 low (69%), with 5yr DMFS \geq 96.0% (better compared to the 14% cut-off).

Conclusions

1) MS was able to re-stratify 16% of patients to a low risk Luminal A-type group with an excellent outcome. 2) Among TN EBC, 5% were classified as Luminal by MS and had an excellent outcome. 3) Albeit limited by low numbers of patients in each subgroup, this study suggest that MS is better correlated with outcome. 4) The observed subtype discrepancies may have an impact on treatment decision making. 5) Centrally assessed Ki67 labeling index of 20% may be the best cut-off for surrogate differentiation between Luminal A and B.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD7-02

Title: Identification of breast cancers with an indolent disease course: 70 gene indolent threshold validation in a Swedish randomized trial of tamoxifen vs. not, with 20 year outcomes

Esserman LJ J, Yau C, Thompson CK K, van't Veer LJ J, Borowsky AD D, Hoadley KA A, Tobin NP P, Nordenskjöld B, Fornander T, Stål O, Benz CC C and Lindström LS S. University of California, San Francisco, San Francisco, CA; University of California, Davis, Davis, CA; University of North Carolina; Karolinska Institutet and University Hospital, Solna, Sweden and Linköping University, Sweden.

Body: Importance: The frequency of cancers with indolent behavior has increased with screening. We asked whether an ultralow risk threshold on a multigene classifier would identify women whose cancers had an indolent course over 2 decades of follow-up, and which features were most predictive of outcome.

Methods: An ultralow risk threshold of the FDA-cleared MammaPrint 70-gene expression score was set to predict long-term absence of breast cancer-specific mortality in the absence of systemic therapy. The Stockholm Tamoxifen (STO) trial conducted between 1976 and 1990, where postmenopausal women with clinically detected node-negative breast cancers <3cm were randomized to receive tamoxifen versus not, was used for validation. Immunohistochemistry markers (n=727) and Agilent microarrays for MammaPrint risk scoring (n=652) were performed from formalin-fixed paraffin-embedded primary tumor blocks. Recursive partitioning was performed using the rpart package in R to select variables and construct a regression tree that best predicts 20-year breast cancer specific survival. Input variables include: age, period of diagnosis, grade, hormone receptor status, HER2 and Ki69 status, 70-gene risk categories (high, low but not ultra, or ultralow), treatment arm and tumor size; and cross-validation was used to select the final regression tree model.

Results: In this trial conducted in the era before mammographic screening, 58% and 42% were MammaPrint low and high risk, respectively, while 15% were above the ultralow threshold. In the tamoxifen treated arm, women with tumors above the ultralow threshold had no deaths at 15 years and their 20-year disease-specific survival rates of 97%; whereas if untreated, their survival rates were 94%. Recursive partitioning identified the ultralow threshold classification as the first primary split in the model. Once the indolent tumors were partitioned out, among women with tumors below the ultralow threshold, the next most prognostic feature was size, where patients with tumors >20mm have worse breast cancer specific survival. The last split in the model divides the patients with tumors ≤20mm into 70-gene high risk vs low but not ultralow risk groups.

Conclusions and Relevance: A threshold of the 70-gene MammaPrint assay can identify patients with indolent disease whose long-term risk of death from breast cancer after surgery alone is exceedingly low. This threshold emerged as the most prognostic variable, followed by tumor size, and mammaprint high vs. low but not ultralow in our recursive partitioning analysis. This suggests that finding indolent tumors early at a small size may not have much impact on patient outcome. Determining the presence of an ultralow risk breast cancer may prevent overtreatment. Conversely, once the indolent tumors are taken out of consideration, both biology and size impact outcome, and finding these tumors at a small size is likely still important and supports screening in this postmenopausal node negative population.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD7-03

Title: A model using grade and hormone receptor staining defines groups at low vs. high risk for distant metastasis: Comparison to the 21-gene recurrence score

Mylander C, Jackson RS, Rosman M, Fujii T, Le Du F, Raghavendra A, Sinha A, Ueno NT T and Tafral L. The Rebecca Fortney Breast Center; Anne Arundel Medical Center, Annapolis, MD; MD Anderson Cancer Center, Houston, TX and Département d'Oncologie Médicale Centre Eugène Marquis, Rennes, France.

Body: Background: The 21-gene recurrence score (RS) combines breast cancer (BC) expression of multiple genes into a single number which is prognostic for BC recurrence. We previously showed that a model using standard pathology data (AAMC Risk Groups) has substantial overlap with RS Risk Groups. The present study compared the recurrence rate of AAMC Risk Groups to that of RS-based Risk Groups as defined by the TAILORx trial and OncotypeDX (ODX) assay.

Methods: From a prospective registry of BC treated at MD Anderson Cancer Center (2/2005 – 5/2015), we selected cases tested with ODX. Cases were excluded for: other cancer in the past 5 years, T4 stage, node positivity, missing grade, missing ER%, ER&PR<1% or HER2 positivity. Three methods were used to categorize distant metastatic risk: ODX and TAILORx Risk Groups were defined using RS, and AAMC Risk Groups were defined using grade and ER/PR level (Tables). For each method, the proportion of patients experiencing metastasis was calculated within Risk Groups.

Results: 1296 cases were included, with a mean follow-up of 3.5 years (25% had ≥ 4.9 years of follow-up). 82 cases (6.3%, 95% CI 5.1 – 7.8%) experienced distant metastasis, with a mean time-to-metastasis of 2.7 years. The proportion of patients experiencing distant metastasis was similar between the AAMC Low Risk Group (1.5%) and the TAILORx (3.2%) and ODX (2.4%) Low Risk Groups.

Table 1: Distant Metastasis in Low Risk Groups

	AAMC Definition (n=329)	TAILORx Definition (n=250)	OncotypeDX Definition (n=704)
Low Risk Definition	Grade 1 & PR ≥1%	RS < 11	RS <18
% with Distant Metastasis	1.5% (95% CI 0.6–3.7%; n=5)	3.2% (95% CI 1.5-6.4%, n=8)	2.4% (95% CI 1.5-3.9%, n=17)
% in Common with AAMC Low Risk Group	100% (329/329)	31.7% (80/250)	33.3% (235/704)

The AAMC Low Risk Group was less than half the size of the ODX Low Risk Group. Of the 5 recurrences in the AAMC Low Risk Group, 1 was ODX Low Risk and 4 were ODX Intermediate Risk; 2 had 1% PR staining. Of the 17 recurrences in the ODX Low Risk Group, 1 was AAMC Low Risk and 5 (all grade 3) were AAMC High Risk; 3 had PR staining < 10%. The proportion of patients experiencing distant metastasis was similar between the AAMC High Risk Group (17.4%) and the TAILORx (16.4%) and ODX (18.2%) High Risk Groups.

Table 2: Distant Metastasis in High Risk Groups

	AAMC Definition (n=235)	TAILORx Definition (n=238)	OncotypeDX Definition (n=148)
High Risk Definition	Grade 3 or ER <20%	RS > 25	RS > 30
% with Distant Metastasis	17.4% (95% CI 12.9-23.0%, n=41)	16.4% (95% CI 12.0-21.8%, n=39)	18.2% (95% CI 12.6– 25.6%, n=27)
% in Common with AAMC High Risk Group	100% (235/235)	56.7% (135/238)	70.3% (104/148)

The number of patients in the AAMC High Risk Group was greater than the ODX High Risk Group.

Conclusions: AAMC Low and High Risk Groups were prognostic of the likelihood of distant metastasis, and performed similarly to TAILORx and ODX Low and High Risk Groups. If RS were omitted for AAMC Low and High Risk cases, 44% [(329+235)/1296] of cases in the present cohort could have been spared ODX testing. The AAMC Risk Groups, using standard pathology data, can reliably identify a large number of patients unlikely to benefit from ODX testing and thus provide substantial cost savings.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD7-04

Title: A novel nomogram model can predict oncotype DX results thus reducing healthcare expenditures

Orucevic A, Bell JL L and Heidel RE E. University of Tennessee Medical Center, Knoxville, TN.

Body: Oncotype DX (ODX) is a commercially available 21-gene recurrence score (RS) assay for breast cancer (BC), which has both prognostic and predictive recurrence value for estrogen receptor-positive (ER+)/HER2-negative (HER2-)/lymph node-negative (LN-) BC. ODX is currently endorsed for us by ASCO, the NCCN, and others for routine guideline application. The RS predicts benefit of adding adjuvant chemotherapy to hormonal manipulation. ODX is costly, a factor which contributes to the test being performed in only ~1/3 of ER+BC in the USA. Disparities of its use in the US and other developed countries were recently published by us and others. The need for finding an accurate widely applicable, readily available surrogate for ODX is apparent. This study serves to develop and validate nomograms which can be used in place of ODX.

National Cancer Data Base (NCDB) analysis from 2010-2012 of ODX tested ER+/HER2-/LN- patients (pts) with 6-50mm tumor size was used to create nomograms for predicting a high or low ODX RS test results. Analysis of NCDB ODX tested pts in 2013 was used for external validation. Age, tumor size, grade, progesterone receptor (PR) status, lymph-vascular invasion (LVI), and the four most frequent BC histologic types were chosen as variables for creating nomograms based on the published methods (JCO, 26(8):1364-70, 2008). Logistic regression was used to generate the scores and predicted probabilities. The predictive accuracy of the regression model was yielded using a Receiver Operator Characteristic (ROC) analysis and model fit was analyzed by plotting the predicted probabilities against the actual probabilities.

Nomograms predicting a high- or low-risk ODX RS test results were created based on results from 27,719 ODX tested pts (2012-2012) and were validated on 12,763 ODX tested pts (2013) (table1).

Table 1.

Clinicopathologic Characteristics	Points assigned for original cohort nomogram (2010-2012; N=27,719)		Points assigned for external validation cohort nomogram (2013; N=12,763)	
	High-risk ODX RS	Low-risk ODX RS	High-risk ODX RS	Low-risk ODX RS
Age	0.1/yr		0.1/yr	
Tumor size	0.8/1mm		0.9/1mm	
Grade 1	0	100	0	100
Grade 2	40	40	37	37
Grade 3	100	0	100	0
PR Positive ≥1%	0	76	0	75
PR Negative <1%	76	0	75	0
LVI Yes	0.5	0	2	0
LVI No	0	0.5	0	2
Invasive ductal carcinoma	0	36	0	32
Invasive lobular carcinoma	36	0	32	0
Invasive ductal and lobular	31	7	30	2
Invasive ductal mixed with other types	7	31	2	30
Maximum points	214		210	
C-index	.89		.89	
p value	<.001		<.001	
95%CI	.88-.89		.88-.90	

Note: actual nomogram to be published in manuscript

Grade and PR status were shown to be the highest predictors for a low- or high-risk ODX RS. The ROC curves for the probabilities of a low- or high-risk RS showed excellent agreement between the nomogram prediction and actual observation with high, acceptable C-indexes-.89 for both internal and external validation cohorts.

We created and validated nomograms that accurately predict for a high- and low-risk ODX score based on the large, National Cancer Data Base which is comprised of >1500 Commission on Cancer accredited facilities. These nomograms may serve to select pts for which further ODX testing is not necessary and may be an excellent surrogate for BC pts for which ODX testing is not available.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD7-05

Title: How 21-gene recurrence score assay is being used to individualize adjuvant chemotherapy recommendations in ER+/HER2 -node positive breast cancer -A national cancer data base study

Peethambaram PP P, Hoskin TL L, Heins CN N, Habermann EB B and Boughey JC C. Mayo Clinic, Rochester, MN.

Body: Introduction: The 21-gene Recurrence Score (RS) assay has been shown in retrospective studies to predict benefit of adjuvant chemotherapy (AC) in node positive (N+) breast cancer (BC) patients (pts). This study evaluates the trends and patterns of use of RS assay in N+, ER+/HER2-breast cancer and the impact of RS on decision to use AC in a real-world multi-institutional database.

Methods: Pts with T1-T4c, N1mi-N3, ER+/HER2- BC diagnosed between 2010 and 2013, included in the National Cancer Data Base were analyzed. Pts who received neo-AC were excluded. Analyses included Cochrane-Armitage tests for trends and multivariable logistic regression assessing factors influencing RS testing and AC recommendations based on RS.

Results: Among 73,049 pts, RS was obtained in 20.6%, increasing from 14.9% in 2010 to 24.4% in 2013 ($p < 0.001$). RS testing was most common in N1mi (43.6%) and N1 (22.0%) and rare in N2/N3 (3.3%) BC. Of the 12,540 BC with quantitative RS results, 61.1% were low RS, 32.3% intermediate RS and 6.6% high RS. AC recommendation was less frequent in pts with RS testing compared to pts not tested (50.4% recommended AC vs 80.9%, $p < 0.001$). In pts with N1mi/N1 BC, recommendation rates for AC were higher with higher RS (see table), however in N2/N3 BC, AC was recommended in the majority (71-88%) regardless of RS. Most pts (>85%) with RS 26-30 or high risk RS were recommended AC regardless of N stage. For pts with low risk RS, recommendation for AC increased significantly with increasing N stage (see table). On multivariable analysis, in pts with low risk RS, AC was more likely to be recommended in those with N1/N2+ stage (OR 2.3 and 9.1 vs N1mi), T2 and T3/T4 tumors (OR 1.3 and 2.2 vs T1 tumors), poorly differentiated tumors (OR 1.6) and younger age (OR 3.4 and 1.7, respectively, for <40 and 40-49 vs 50-59) (all $p < 0.001$). Among pts with RS 18-25, AC was more likely to be recommended in those with higher tumor grade, younger age but the effect of N stage was less pronounced. Histology (IDC vs ILC) did not influence AC recommendation in any RS subset.

Path N Stage	RS Risk Group			
	Low Risk	Intermediate Risk		High Risk
	RS<18	RS 18-25	RS 26-30	RS>30
N1mi AC No	1,854 (75.9%)	389 (36.9%)	36 (14.6%)	16 (7.0%)
N1mi AC Yes	590 (24.1%)	666 (63.1%)	210 (85.4%)	212 (93.0%)
N1 AC No	3,000 (60.6%)	699 (33.0%)	75 (14.8%)	33 (6.0%)
N1 AC Yes	1,954 (39.4%)	1,422 (67.0%)	431 (85.2%)	518 (94.0%)
N2/N3 AC No	76 (29.0%)	17 (16.7%)	3 (12.5%)	7 (14.9%)
N2/N3 AC Yes	186 (71.0%)	85 (83.3%)	21 (87.5%)	40 (85.1%)
p-value	<0.001	<0.001	0.88	0.16

Conclusions: RS was obtained in about one fifth of pts with N+ ER+/HER2- BC, predominantly for N1mi and N1 disease. Overall, RS testing decreased frequency of recommendation of AC in N+ BC pts. The RS influenced use of AC particularly in N1mi and N1 pts, likely avoiding overtreatment of those with low risk RS and RS 18-25. Prospective data regarding RS to direct AC in N+ BC are awaited.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD7-06

Title: SEER study of breast cancer-specific mortality in patients with poorly differentiated tumors treated based on recurrence score results

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Body: Introduction: The SEER Program of the NCI is an authoritative source of cancer incidence and survival statistics. Linking the 21-gene assay Recurrence Score® (RS) results to the SEER Registries (N=44,825) demonstrated very low 5-year breast cancer-specific mortality (BCSM) with RS <18 across many key clinical-pathological subgroups, such as age, nodal status, tumor grade, and size (*npj Breast Cancer*. 2016;2:16017). Given the large sample size and specific interest in outcomes as a function of tumor grade, further stratification of patients with poorly differentiated tumors was performed to determine BCSM when examined by both tumor grade and tumor size.

Methods: Patients were eligible if node negative (N0), HR+, HER2-negative (by RT-PCR), had no prior malignancy, had poorly differentiated (G3) tumors, and were diagnosed between Jan 2004 (test available in Jan 2004) and Dec 2011 (SEER survival updated through 2012). BCSM was defined according to pre-existing robust methodology (*J Natl Cancer Inst*. 2010;102:1584). RS was categorized according to the cutpoints of 18 and 31 established in the NSABP B-14 study. Five-year BCSM was estimated using actuarial methods.

Results: Among 6,666 eligible patients with G3 tumors, 4,683 had tumors ≤2 cm and 1,983 had tumors >2 cm. Median age was 57 years; 99.1% were female. Median follow-up was 39 months. The proportion of patients with RS <18 was 29% among those with tumors ≤2 cm and 25% among those with tumors >2 cm, somewhat lower than the overall population. For RS <18, 5-year BCSM was 0.3% (G3; ≤2 cm) and 1.4% (G3; >2 cm); reported chemotherapy use was 10% and 16%, respectively. 5-year BCSM for all groups are provided in Table. An additional year of BCSM follow-up in N0 G3 disease, as well as results for patients with node positive (micrometastases or 1-3 positive nodes) G3 disease, will be available for presentation.

	N0, G3 tumor, RS <18		N0, G3 tumor, RS 18-30		N0, G3 tumor, RS ≥31	
Tumor size	N	5-y BCSM (95% CI)	N	5-y BCSM (95% CI)	N	5-y BCSM (95% CI)
≤2 cm	1362	0.3% (0.1%, 1.2%)	2148	2.1% (1.3%, 3.3%)	1173	2.9% (1.8%, 4.7%)
>2 cm	486	1.4% (0.4%, 4.6%)	851	4.6% (2.7%, 7.8%)	646	9.0% (6.0%, 13.4%)

Conclusions: Although patients with poorly differentiated tumors have worse prognosis in general, the RS identifies a sizable proportion of patients who can expect good outcomes without chemotherapy and its associated toxicity.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD7-07

Title: Discovery of molecular predictors of late breast cancer specific events (BCSE) in ER+, node+ breast cancer – new transcriptome expression whole gene analysis of the phase III adjuvant trial SWOG S8814

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Body: BACKGROUND: Unique genes and pathways were identified for prognosis on tamoxifen (T, 5 yrs) and prediction on CAF-T vs T in S8814 using whole transcriptome RNA-Seq from archival FFPE tissue. (Albain, et al; Cherbavaz, et al; SABCS 2015) Discovery was robust for early DFS events but sparse for late. The aims of this new analysis were to 1) utilize a new endpoint BCSE for gene discovery of late events, prognosis and prediction and 2) add intronic counts to the previous exonic results to define whole genes impacting on late BCSE.

METHODS: Charts of patients (pts) on CAF-T (212) vs T (142) were reviewed to define the BCSE endpoint (local/regional, contralateral, distant). Deaths without BC were treated as competing risks. BCSE models (including metagenes) of late prognosis and prediction used cumulative incidence functions. Consolidated intronic regions counts within genes were added to exonic regions counts. Using these “whole gene” (WG) counts, association of gene expression with time to BCSE was assessed by Cox regression. A multiple WG score (MWGS) for BCSE prognosis beyond 5 yrs (to 12.5 yrs) was constructed and evaluated for 1-3 and 4+ node (N) groups. False discovery rate was controlled at 10%.

RESULTS: More exons and WG were discovered for prognosis on T alone over 12.5 yrs with the BCSE endpoint than DFS. For prognosis of late BCSE after 5 yrs, more genes were discovered using WG (n=111) than by exons (n=9). There were significantly fewer genes for late BCSE on CAF-T (8, WG; 0, exons). The functions of WG prognostic for late BCSE were: cell cycle/proliferation-26 genes, chromosome segregation/mitotic spindle-22, DNA repair/maintenance-10, transcriptional/translational control-5, cell adhesion/migration-4, immune-3, diverse/unknown-32 and growth factor/hormone receptor signaling-9 (this group was only found by WGs, not exons). Of these 111 WG, a MWGS prognostic for late BCSE on T used 57 previously discovered genes pre-specified for this analysis. Probability of BCSE beyond 5 yrs for low vs high MWGS was 8% vs 21% in N1-3+ and 17% vs 42% in N4+. Late prognosis on T differed by low vs high risk defined in a metagene model: cumulative BCSE at year 10 was 0% vs 47% (low vs high risk, p=0.001). Prediction of 10-yr incidence of BCSE varied by risk level by treatment in a metagene model: low risk- CAF-T=47%, T=0% (p=0.045); high risk- CAF-T=35%, T=45% (p=0.027).

CONCLUSIONS: Gene discovery for prognosis of late BCSE is enhanced with a novel WG transcriptome expression approach. Use of chemotherapy (CT) before T significantly attenuated gene discovery, so that molecular tools for decisions on extending endocrine therapy (ET) may not be reliable in a setting of prior CT. Some pts on ET for 5 yrs may not require either longer ET or CT, given a N+ cohort was defined with no BCSE observed over 12.5 yrs. For prediction of CT benefit, CAF-T appeared to be inferior to T in a low risk metagene model for BCSE. In sum, these results add more evidence that ET alone may be sufficient (perhaps better) in select N+ settings. Validation in SWOG S1007 (RxPONDER) is planned.

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2016 San Antonio Breast Cancer Symposium

Publication Number: PD8-01

Title: Race and age differences in PAM50 biomarker status in the Carolina breast cancer study

Troester MA A, Sun X, Allott EH H, Kit C-K, Thorne L, Mathews M, Cohen SM M, Geradts J, Kirk E, Li Y, Hu Z, Robinson W, Hoadley KA A, Reeder-Hayes K, Earp S, Olshan AF F, Carey LA A and Perou CM M. University of North Carolina at Chapel Hill and Dana Farber Cancer Institute.

Body: Background: African American breast cancer patients have lower relative frequency of hormone receptor (HR)-positive/HER2-negative disease and higher subtype-specific mortality. However, few population-based studies have RNA-based subtyping data, and racial differences in the biology of HR-positive/HER2-negative tumors are not well understood.

Methods: Using data and biospecimens from the Carolina Breast Cancer Study (CBCS) Phase 3 (2008-2013), we classified approximately 1,000 invasive breast cancers according to PAM50 subtype and two risk of recurrence scores (ROR-P and ROR-PT). Relative frequency of Luminal A, Luminal B, Her2-enriched, and Basal-like subtypes and ROR scores (low/medium/high) were compared by race (blacks vs. whites) and age (≤ 50 years vs. > 50 years), overall and among HR-positive/HER2-negative cases.

Results: Black women of all ages had significantly higher relative frequency of Basal-like breast cancer (36 and 31% in blacks vs. 18 and 15% in whites; younger and older, respectively) and lower frequency of Luminal A breast cancer (26 and 34% in blacks vs. 43 and 52% in whites; younger and older, respectively). Frequency of Luminal B and HER2-enriched breast cancer did not vary by race or age. Among clinically HR-positive, HER2-negative cases, Luminal A subtype comprised only half of the cases among black women, and was significantly less common than among white women (51% vs 60% in whites, $p < 0.05$). Black women with HR-positive/HER2-negative disease also had significantly higher ROR scores (ROR-P medium or high 82% vs. 66% in whites, $p = 0.01$; ROR-PT medium or high 85% vs. 69% in whites, $p < 0.01$).

Conclusions: Multi-gene assays highlight disparities in frequency of aggressive, poorer prognosis tumor subtypes and implicate differences in tumor biology as an important contributor to mortality disparities among HR-positive/HER2-negative patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD8-02

Title: Tumor subtype and survival differences between Hispanic and non-Hispanic white breast cancer patients in the California cancer registry

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Body: Background: Prior studies show that Hispanic breast cancer patients are more likely than non-Hispanic white (NHW) women to be diagnosed with triple negative tumors but data on other subtypes are limited. Published data on survival differences between Hispanics and NHW breast cancer patients are inconsistent and vary depending on the covariates included in the multivariate models. We assessed differences in the distribution of the major tumor subtypes of breast cancer, as well as subtype-specific survival, between Hispanic and NHW patients according to nativity, and age and stage at diagnosis.

Methods: We used data from the population-based California Cancer Registry to include female invasive breast cancer cases diagnosed between 2004 and 2013 with follow-up through December 31, 2013, resulting in 90,236 total cases (69,693 NHW and 20,543 Hispanics). Tumor subtypes were classified into four categories: hormone receptor positive and HER2 negative (HR+/HER-), HR+/HER2+, HR-/HER2+, and triple negative (HR-/HER2-). Logistic regression was used to estimate differences in distribution of subtype between Hispanic and NHW women. Cox proportional hazard models were used to estimate differences in survival for Hispanics and NHWs by subtype, adjusting for clinical and sociodemographic characteristics.

Results: Compared to NHW patients, Hispanic women were more likely to be diagnosed with tumors that were HR+/HER2+ (OR=1.22; 95% CI, 1.16-1.29), HR-/HER2+ (OR=1.37; 95% CI, 1.29-1.47), and triple negative (OR=1.27; 95% CI, 1.21-1.34) than HR+/HER2-. Foreign-born Hispanics had a higher odds of having HER2+ than HR+/HER2- tumors compared to NHW women (OR=1.29 for HR+/HER2+ and OR=1.50 for HR-/HER2+); these differences were less pronounced among U.S.-born Hispanics (OR=1.04 for HR+/HER2+ and OR=1.16; 95% CI, 1.06-1.26 for HR-/HER2+). In age-adjusted models, Hispanic women had higher breast cancer mortality than NHW women (HR=1.23; 95% CI, 1.17-1.30), which was consistent across all tumor subtypes. However, the mortality differences disappeared after adjustment for clinical, sociodemographic characteristics, and marital status.

Conclusions: Hispanic women were more likely than NHWs to be diagnosed with triple negative breast cancer as well as tumors overexpressing HER2 than HR+/HER2- tumors. This pattern held true when stratified by nativity, although higher ORs for HER2+ tumors among foreign-born than U.S.-born Hispanic women were observed. Within each subtype, Hispanics had 20%-30% higher mortality than NHW, which appeared to be explained by a combination of sociodemographic and clinical factors.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD8-03

Title: Differences in the mutational landscape in African Americans and Caucasians with triple negative breast cancer

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Body: Background-Triple negative breast cancer (TNBC) occurs at a higher frequency in African American (AA) premenopausal women compared with Caucasians. It is unclear if the biology and clinical outcome of TNBC is different in AA versus Caucasians. In this study, we sought to evaluate differences in the molecular pathology of TNBC in a large cohort of AA and Caucasians.

Methods-Using publicly available data from The Cancer Genome Atlas, we identified all patients with TNBC who had information on race. We analyzed the differences in clinical characteristics and tumor somatic mutations by race from whole exome sequencing using student's t test or one-way ANOVA for continuous variables and chi square or Fisher's test for categorical variables. Exome sequencing was performed with coverage of $\geq 70\%$ and at a depth of 20x.

Results-A total of 1104 primary breast cancer patients were identified, of which 178 (16%) had TNBC. Overall, TNBC was more frequent in AA than in Caucasians (33.3% versus 14.9%; p value < 0.001). More AA than Caucasians were classified as basal-like from PAM50 gene expression analysis (34.8% versus 16.1%; p value 0.001). No differences in the TNBC cohort were observed, 91% of AA were basal-like versus 81% of Caucasians (p value 0.842). Median tumor somatic mutation counts were higher in AA (39.5) versus Caucasians (34) (p value 0.022). However, no racial differences in the mutation counts in TNBC were observed (AA=56, Caucasians=60, p value 0.399). Somatic mutation analysis revealed racial differences in specific high prevalence ($> 5\%$) genes in all patients- [*TP53* alterations: 46% in AA versus 27% in Caucasians; p value < 0.001 , *PIK3CA* alterations: 23% in AA versus 34% in Caucasians; p value 0.019, and *MLL3* alterations: 12% in AA versus 6% in Caucasians; p value 0.028]. The TNBC patients did not have any specific high prevalence ($> 5\%$) genes associated with racial differences. AA with breast cancer had a shorter time to progression (TTP) (hazard ratio 1.62, p value 0.014) and worse disease free survival (DFS) than Caucasians. However, racial difference in TTP or DFS were not observed in the TNBC patients.

Conclusion-The mutational landscape of breast cancer may be different between AA and Caucasians, but appears to be similar in both races in the cohort with TNBC. Our findings have 2 implications: racial disparities in breast cancer may largely be due to differences in those with hormone receptor positive disease, and secondly, the higher frequency of TNBC in AA is unlikely due to differences in molecular features, but other modifiable factors.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD8-04

Title: Racial differences in the molecular landscape of breast cancer

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Body: Background:African Americans (AA) have a higher mortality associated with breast cancer (BC) when compared to Caucasians (CC). This has been attributed to diverse factors that include access to care, reproductive factors and different somatic genomic profiles. We aimed to compare the racial mutational landscape of 565 BC samples.

Methods:DNA from formalin-fixed paraffin-embedded samples was sequenced using the Illumina NextSeq (Agilent SureSelect XT, 592 gene selected based on COSMIC database) and MiSeq (TruSeq, 47 gene) for mutation and gene amplification analyses. Protein expression was evaluated by Immunohistochemistry (IHC). The exome aggregation consortium database was assessed for known ethnicity associations.

Results:Tumor samples from 118 AA and 447 CC female patients were included in this analysis. AA were younger (median age 56 vs58y, p0.005) and had higher proportion of triple negative BC (TNBC) (32% vs17%, p0.001). 50.3% of the samples were obtained from primary tumors and the remainder from metastatic sites. This was similar in AA and CC (48.8% vs51.8% primary tumors, pNS). The two genes with highest mutation prevalence were TP53 and PIK3CA. AA had fewer PIK3CA mutations (14.7% vs28.2%, p0.03). Within HR+/HER2+ and HR+/HER2- subtypes there was a similar trend in the number of PIK3CA mutations but it was no longer significant. The remainder mutation analysis did not differ between races. In terms of protein expression there were significant differences in the androgen receptor(AR), RRM1, EGFR and TS expression (table). AR positivity defined as $\geq 10\%$ was less frequent in AA (40.0% vs60.4%, p0.0001 and when adjusted for age, p0.005) and associated with PIK3CA mutations in both AA and CC (p0.01 and p0.007). AR expression in TNBC was positive in 17.8% of CC and 5.4% of AA (pNS). Copy number variation (CNV) data assessed by NextGen revealed significantly higher gene copy number in AA compared to CC in CCND1 (16% vs4%, p0.04), FGF19 (16% vs1.3%, p0.01) and FGF4 (16% vs3%, p0.02). When only TNBC was considered, RRM1, TOPO1 and TUBB3 expression was significantly higher in AA than CC (table) and there were no differences in the mutational analyses. Evaluation of other BC subtypes (HER2, HR positive) is currently underway.

Conclusions:In this large cohort of AA who underwent genomic profiling there were relatively few differences in the mutation analysis compared to CC. The only significant difference seen was the lower number of PIK3CA mutations in AA, which had been previously reported in a cohort of 105 AA from the TCGA data (Keenan et al. JCO 2015). Protein expression by IHC revealed lower expression of AR in AA, even after adjustment for age, which could have therapeutic implications. Some of the racial differences found in the molecular landscape of BC including PIK3CA mutations, AR, EGFR expression and CNV may contribute to a more aggressive tumor biology in AA.

Protein expression by IHC

	BC			TNBC		
	AA %	CC %	p	AA %	CC %	p
HER2/neu	9	12	ns	0	0	ns
AR	40	60	0.00	5	18	0.07
ER	44	62	0.00	0	0	ns
PR	35	46	0.09	0	0	ns
EGFR	30	16	0.03	60	50	ns
ERCC1	33	46	0.06	36	26	ns
MGMT	61	63	ns	65	71	ns
PD-1	50	34	0.06	65	48	ns

PD-L1	0	6	ns	0	11	ns
PGP	7	6	ns	10	15	ns
PTEN	56	58	ns	42	47	ns
RRM1	39	27	0.04	59	27	0.01
TLE3	50	59	0.10	43	57	ns
TOP2A	71	64	ns	86	47	0.08
TOPO1	63	58	ns	69	46	0.03
TS	44	30	0.01	63	49	ns
TUBB3	55	41	0.06	77	51	0.03

2016 San Antonio Breast Cancer Symposium

Publication Number: PD8-05

Title: Comparative analysis of the genomic landscape of breast cancers from women of African and European ancestry

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Body: Objectives: Paucity of data on populations of African Ancestry in clinical trials continues to limit our ability to design and implement innovative solutions to narrow the breast cancer survival gap amongst Africans, African Americans, and European Americans. We have developed a cross-continent research infrastructure to examine the spectrum of genomic alterations in breast tumors from West Africa and subsequently, to compare them to tumors from African American women and women of European Ancestry in The Cancer Genome Atlas (TCGA) database.

Methods: Consecutive women with breast cancer presenting for treatment at the University College Hospital, Ibadan and at Lagos State University Teaching Hospital, Lagos, Nigeria gave informed consent and were recruited to the West African Breast Cancer Study (WABCS) between 2013-2016. Tumor-normal pairs were subjected to exome and/or high-depth (90x) genome sequencing. High confidence somatic mutations (substitutions, insertions/deletions and structural variants) were obtained by using multiple variant callers. Furthermore, 1,089 exomic and 80 genomic breast tumor-normal pairs from TCGA were harmonized with WABCS samples, resulting in a cohort of 147 West Africans (147 exome; 40 genome), 154 African Americans (154 exome; 31 genome), and 776 Caucasians (776 exome; 43 genome).

Results: Across the exomes, genes commonly altered in breast cancer in TCGA are also altered in women of African ancestry, but the mutational spectrum is quite different, demonstrating overrepresentation of tumors with aggressive phenotypes. Overall, *TP53* (65%), *ERBB2* (27%), and *GATA3* (17%) showed statistically significant higher alteration frequencies in West Africans and African Americans. In contrast, *PIK3CA* (24%) was less frequently mutated. Of note, *GATA3* mutation was statistically significantly more frequent in Nigerians (39%) and African Americans (16.7%) compared to Caucasians (10.5%), in ER-positive cancers. Analysis on Structural Variants (SV), on the other hand, has shown that the genome-wide SV counts among three populations are comparable in ER-negative cancers, while Nigerians have significantly more SV counts compared to African Americans ($P=0.0013$) or European Americans ($P=2.9 \times 10^{-5}$) in ER-positive cancers. Similarly, genome-wide substitution patterns in ER+ and ER- cancers varied widely by race/ethnicity. In ER- cases, West Africans carried the highest proportion of canonical APOBEC-associated substitutions, particularly C>T transitions. Conversely, European Americans with ER+ disease showed a higher proportion of C>T than both West Africans (Welch t-test $P = 0.044$) and African Americans (Welch t-test $P = 0.011$). Mutation signature analyses highlighted multiple APOBEC signatures, with notable contribution differences across ancestry and ER status. A signature likely corresponding to DNA damage repair correlated with the proportion of genetic ancestry, being most prevalent in European Americans and least common in Nigerians, particularly in ER-negative cancers, with African Americans showing a degree of this signature's contribution in between the two populations (linear model adjusted for age, $P=1.0 \times 10^{-10}$).

Conclusions: Overall, our data suggests mutation spectra differences in across race/ethnicity and geography. Identification of molecular characteristics such as higher rates of *HER2* enriched tumors and higher rates of *GATA3* mutations in ER positive tumors are beginning to reveal the genomic basis of race-associated phenotypes and outcomes in breast cancer. Population differences in frequency and spectrum of mutations should now inform the design of innovative clinical trials that improve health equity and accelerate Precision Oncology care in diverse populations.

2016 San Antonio Breast Cancer Symposium

Publication Number: PD8-06

Title: Multiple-gene panel testing for hereditary cancer risk reveals a racial/ethnic disparity in genetic information

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Body: Background: Multiple-gene germline sequencing panels are increasingly used to evaluate hereditary cancer risk. However, little is known about the results of such testing in racially/ethnically diverse populations.

Methods: Patients who presented to the Stanford University Clinical Cancer Genetics Program from 1/1/2013 to 12/31/2015 and underwent multiple-gene panel testing were included in the cohort (N=1,483). Information on demographics, personal and family history of cancer, and tumor characteristics was collected at time of clinic visit. Odds ratios and 95% confidence intervals were calculated for mutation status by different patient and tumor characteristics, including race/ethnicity. Results were compared using the chi-square test and considered significant if $P < 0.05$ after Bonferroni correction for multiple hypothesis testing.

Results: Most patients (92%) were female. Patients were 51% Non-Hispanic White (NHW), 19% Asian, 14% Hispanic, 10% Ashkenazi Jewish, 5% other, and 1% unknown. Eighty-nine genes were tested in at least one patient; panel size ranged from six to 62 genes, with a median of 25 genes. The frequency of pathogenic or likely pathogenic mutations was 15% for any panel-tested gene, 5.5% for *BRCA1/2*, and 5.3% for other breast cancer-associated genes; mutation frequencies were similar between NHW, Asian, Hispanic, and Ashkenazi Jewish patients. Variables significantly associated with the carriage of a pathogenic *BRCA1/2* mutation included personal or family history of ovarian cancer and personal history of triple-negative breast cancer (TNBC). No variable was significantly associated with the presence of a pathogenic mutation in the other breast cancer genes. The odds ratios for carrying a *BRCA1/2* mutation with personal history of ovarian cancer (4.1 [2.2-7.3]), family history of ovarian cancer (3.1 [1.9-4.9]), and TNBC relative to other breast cancer subtypes (6.0 [2.9-12.4]) did not differ significantly between NHWs and non-Whites. The frequency of variants of unknown significance (VUS) in any panel-tested gene was higher in non-Whites (36%) than in NHWs (27%) ($P=2E-4$), with the highest odds ratio relative to NHWs among Asians (2.0 [1.5-2.7], vs 1.3 [0.9-1.7] among Hispanics and 1.2 [0.8-1.7] among Ashkenazi Jews). The odds ratio of finding a VUS for non-Whites compared to NHWs was similar for any panel (1.5 [1.2-1.9]), for *BRCA1/2* (1.3 [0.8-2.3]), and for other known breast cancer-associated genes (1.5 [1.1-1.9]).

Conclusions: In this diverse cohort tested for hereditary cancer risk with multiple-gene panels, frequencies of pathogenic mutations were similar between racial/ethnic groups. By contrast, frequencies of VUS were significantly higher among non-Whites compared to NHWs. This higher VUS rate renders multiple-gene panel testing less informative for non-White patients. Efforts toward VUS re-classification, particular among non-Whites, are urgently needed to address this genetic information disparity.

2016 San Antonio Breast Cancer Symposium

Publication Number: S5-01

Title: DCIS biological risk profile predicts risk of recurrence after breast conserving surgery in a Kaiser Permanente NW population

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Body: Background: Patients with DCIS and their physicians need tools that provide better information about the individual patient's biological risk profile to help make treatment decisions. Prelude and the Kaiser Permanente Northwest Center for Health Research (KPCHR) validated a biological risk signature based test to assess ipsilateral breast event (IBE) risk after breast conserving surgery (BCS) with radiation (+RT) or without radiation therapy (-RT).

Methods: The Prelude DCIS test was independently validated in a retrospective cohort from the Kaiser Permanente Northwest (KPNW) integrated healthcare system in patients diagnosed with DCIS from 1990- 2007 and treated with BCS±RT(n=608). KPCHR performed central pathology review to identify patients meeting study eligibility criteria with formalin fixed paraffin embedded (FFPE) tissue samples (n=475); KPCHR also reviewed medical records to collect patient, treatment, and outcome data. FFPE patient samples were provided to Prelude for testing. REMARK guidelines were followed.

A panel of biomarkers (HER2, PR, Ki-67, COX2, p16/INK4A, FOXA1 and SIAH2) were assayed by the Prelude CLIA lab and scored by board-certified pathologists (n=455). Prelude's DCIS test was executed independently using biomarker and clinicopathologic data while blinded to patient outcome data. The risk results were provided to KPCHR under a Data Transfer Authority. KPCHR biostatisticians executed a predefined and co-developed statistical analysis plan. IBE rates were assessed using Kaplan-Meier survival analysis. Hazard ratios (HR) were determined using Cox proportional hazards analysis, with RT as a covariate.

Results: The Prelude DCIS test score was statistically associated with total IBE as a continuous linear variable (0-10 unit scale) on a per unit basis, HR of 1.12, 95% CI [1.03,1.23], p=0.01. The DCIS test score (0-10) corresponded to recurrence risks ranging from 10% to 42% (≤ 2 , > 7) for patients treated with BCS-RT and ranging from 4% to 11% (≤ 2 , > 7) for patients treated with BCS+RT. Patients treated with BCS ±RT with an elevated test score (≤ 3 vs > 3) had a higher recurrence risk, n=455, HR=1.87 [1.03 - 3.38], p=0.04. In patients treated with BCS-RT in this sample, patients with a higher DCIS signature had an elevated recurrence risk, n=78, HR=2.37, 95% CI [0.82, 6.85], p=0.11. The 10-year contralateral breast event rate was 4%, 95% CI [2%, 6%]. Median follow-up time was 10.4 years.

Discussion: Patients diagnosed with DCIS and treated with BCS ±RT, were stratified into clinically relevant low and elevated risk groups (≤ 3 vs > 3) in an independent validation of the Prelude DCIS test. Patients in the elevated risk group had substantially higher likelihood of 10-year total IBE. The number of patients treated with BCS -RT was limited and while the stratification by risk group for BCS -RT was in the expected direction, it did not reach statistical significance. Two additional validation studies are scheduled to be completed in 2016.

10-YEAR IBE RISK

	BCS -RT			BCS +RT		
	Risk, [95% CI]	Prevalence	N	Risk, [95% CI]	Prevalence	N
Baseline Total Risk	20%, [12%, 32%]	100%	78	8%, [5%, 11%]	100%	377
Low Risk Group (≤ 3)	10% [3%, 29%]	53%	41	5%, [2%, 10%]	40%	149
Elevated Risk Group (> 3)	30%, [17%, 51%]	47%	37	10%, [6%, 15%]	60%	228

2016 San Antonio Breast Cancer Symposium

Publication Number: S5-02

Title: Scalp cooling alopecia prevention trial (SCALP) for patients with early stage breast cancer

Nangia JR R, Wang T, Niravath PA A, Otte K, Osborne CR R, Papish S, Holmes FA A, Abraham J, Goldfarb S, Courtright JG G, Paxman RJ J, Rude MB B, Hilsenbeck SG G, Osborne CK and Rimawi MF F. Baylor College of Medicine, Houston, TX; US Oncology; Summit Medical Group-Morristown; Cleveland Clinic; Memorial Sloan Kettering Cancer Center and Paxman Coolers LTD.

Body: Background

Adjuvant chemotherapy decreases the risk of breast cancer recurrence. However, it is associated with distressing side effects, including alopecia. Women consider chemotherapy-induced alopecia as one of the most severe side effects. In many countries, scalp cooling is used to prevent chemotherapy-induced alopecia. It causes cutaneous vasoconstriction, which reduces blood flow to hair follicles during peak plasma concentrations of the drug and may reduce cellular uptake of these agents. It also results in reduced biochemical activity, which makes hair follicles less susceptible to the damage of chemotherapy. There are no randomized trials assessing this approach with modern scalp cooling and success rates in non-randomized trials have varied.

Methods

We conducted a multi-center randomized non-blinded controlled prospective trial to evaluate the safety and efficacy of the Orbis Paxman Hair Loss Prevention System (OPHLPS) in reducing chemotherapy-induced alopecia. Women with stage I-II breast cancer planned to receive neoadjuvant or adjuvant anthracycline- or taxane- based chemotherapy for at least four cycles were eligible. Participants were randomized in a 2:1 ratio to scalp-cooling or no cooling. Scalp-cooling was done using the OPHLPS 30 minutes prior to, during and 90 minutes after each chemotherapy. The primary efficacy endpoints were hair preservation, defined as CTCAE 4 alopecia <2 (assessed by an independent and blinded evaluator), and device safety. We planned to enroll 235 patients to provide 85% power to detect a 20% difference in hair preservation (i.e. 15% with no cooling and 35% with scalp-cooling). Secondary endpoints included wig/scarf use and quality of life assessed by the EORTC QLQ-30, HADS and BIS. Participants will be followed for 5 years post-study for time to first recurrence, overall survival, site of first recurrence, and incidence of isolated scalp metastasis. One interim analysis was planned to allow the study to stop early for efficacy (superiority) after 95 and 47 patients were enrolled to cooling or no cooling, respectively, and have been evaluated for the primary endpoint. To maintain the overall type 1 error rate, an O'Brien-Fleming spending function has been used to set the superiority boundaries (interim boundary was calculated as $p=0.0061$ (or $Z=2.509$)).

Results

This is the first prospective randomized trial with modern scalp cooling in the world. At the time of the interim analysis, 95 patients in the cooling group and 47 patients in the no cooling group were evaluable and had completed 4 cycles of chemotherapy. Among them, 48 (50.5%) out of 95 in the cooling group and 0 (0%) out of 47 in the no cooling group had hair preservation. The one-tailed p-value from the Fisher's exact test is <0.0001, which crosses the superiority boundary ($p=0.0061$). Thus, on 9/26/2016, the DSMB decided to stop the study early and release the results. Our trial shows that scalp cooling using OPHLP is highly effective in hair preservation and should become available for patients who receive chemotherapy for early breast cancer. These data and secondary endpoint analysis data will be presented. We are seeking FDA approval for the OPHLPS.

2016 San Antonio Breast Cancer Symposium

Publication Number: S5-03

Title: Menopausal symptoms as predictors of long-term adherence in the International breast cancer intervention study (IBIS-1)

Smith SG G, Sestak I, Forbes J, Howell A and Cuzick JJ J. Queen Mary University of London, London, United Kingdom; The University of Newcastle Australia, Newcastle, Australia and The University of Manchester, Manchester, United Kingdom.

Body: Tamoxifen reduces the risk of breast cancer among women at increased risk of the disease. The effectiveness of the drug is dependent on its appropriate use for the duration of therapy. Adherence for the full course of preventive therapy ranges from 60-80%. Early discontinuation is largely attributed to side-effects, but no studies have validated this using 5-year follow-up data. We assessed the role of menopausal side-effects on long-term adherence in the UK arm of the International Breast Cancer Intervention Study (IBIS-1).

In the IBIS-1 trial, 4279 women in the UK were randomised to placebo vs. tamoxifen (20mg/day). A total of 292 were excluded (breast cancer, death, or other cancer), leaving 3987 to be included in the analysis (2000 placebo arm, 1987 tamoxifen arm). Adherence was calculated using data from six monthly clinical visits during the trial. Adherence (<4.5 years, ≥4.5 years) was calculated using the Kaplan-Meier estimator. Our main objective was to investigate the effect of menopausal side effects experienced during the first six months on adherence overall and by treatment arm. All analyses were adjusted for age, Tyrer-Cuzick risk, smoking, hormone replacement therapy use, menopausal status, baseline symptoms, and treatment. Overall, 66.8% of women were adherent for at least 4.5 years (placebo: 71.5% vs. tamoxifen: 62.1%, $p < 0.001$). Highest drop-out rates were found within the first 12 months of follow-up, and a significant difference in adherence between treatment arms was observed thereafter ($p < 0.05$). A low number of women experienced nausea/vomiting (5.0%) and headaches (7.0%), while higher proportions reported gynaecological symptoms (irregular bleeding/vaginal dryness/vaginal discharge, 13.8%) and hot flushes (31.5%). Women reporting nausea/vomiting (OR=1.82 [95% CI, 1.35-2.47], $p < 0.001$) and headaches (OR=1.41 [1.08-1.84], $p = 0.01$) were significantly more likely to be non-adherent at 4.5 years. Long-term adherence was not significantly affected for those who reported hot flushes (OR=1.10 [0.94-1.30], $p = 0.2$) or gynaecological symptoms (OR=1.18 [0.99-1.41], $p = 0.07$) compared with their counterparts. Nausea/vomiting was significantly associated with non-adherence in both the placebo (OR=1.82 [1.16-2.87], $p = 0.009$) and tamoxifen (OR=1.84 [1.22-2.76], $p = 0.004$) arms. Headaches were associated with non-adherence in the placebo arm (OR=1.70 [1.16-2.50], $p = 0.006$), while gynaecological symptoms were only significant in the tamoxifen arm (OR=1.30 [1.05-1.63], $p = 0.02$). The majority of side effects were of mild or moderate severity and we observed significant trends for lower adherence with increasing severity for all symptoms ($p < 0.05$ to < 0.001).

In the IBIS-1 trial, we observed a significant effect of common menopausal symptoms on long-term adherence. These effects were largely similar between tamoxifen and placebo arms, suggesting women are attributing age-related menopausal symptoms to preventive therapy. To ensure women experience the full benefit of preventive therapy, interventions are required to support the management of menopausal symptoms and to promote adherence. The higher rate of drop-out in the early months of the trial suggests early intervention may be an effective way to promote long-term adherence.

2016 San Antonio Breast Cancer Symposium

Publication Number: S5-04

Title: Low-fat dietary pattern and breast cancer overall survival in the women's health initiative dietary modification randomized controlled trial

Chlebowski RT T, Aragaki AK K, Thomson CA A, Anderson G, Manson JE E, Simon MS S, Rohan TE E, Snetselar LG G, Lane D, Barrington WE E, Vitolins M, Womack C, Qi L, Hou L, Thomas F and Prentice RL L. Los Angeles BioMedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; Fred Hutchinson Cancer Research Center; University of Arizona; Brigham and Women's Hospital; Karmanos Cancer Institute; Albert Einstein College of Medicine; Stony Brook University; University of Texas Health Science Center; University of California at Davis, Davis; University of Iowa, Iowa City/Davenport; Northwestern University Feinberg School of Medicine and University of Tennessee Health Science Center.

Body: Introduction: Among 48,835 postmenopausal women randomized in the Women's Health Initiative Dietary Modification (WHI DM) primary prevention trial, 1,767 women were diagnosed with breast cancer during the 8.3 years of dietary intervention. While differences were not statistically significant, there were fewer breast cancers diagnosed in the low fat dietary group women (HR 0.92 95% CI 0.84-1.01, P=0.09) with somewhat lower breast cancer mortality (HR 0.77 95% CI 0.48-1.22) than seen in control group women (JAMA 2006; 295:629). These findings were recently updated, and after 10.9 years (mean) post-diagnosis follow-up, breast cancer overall survival among these 1,767 women measured from diagnosis was greater in the dietary group (10 year survival, 82% vs 78%, 168 (2.24%) versus 319 (2.71%) deaths; HR 0.80 95% CI 0.66-0.97, P=0.02) (AACR Annual Meeting 2016, abstract CT0433, Clinical Trials Plenary Session). We now report low-fat dietary pattern influence on breast cancer overall survival in subgroups defined by breast cancer characteristics.

Methods: The WHI DM trial, conducted at 40 US clinical centers, from 1993-1998 enrolled 48,835 postmenopausal women, aged 50-79, without prior breast cancer, with normal mammogram and dietary fat intake >32% of total energy. Participants were randomly assigned to a dietary intervention group (40%, n=19,541) with goals of fat intake reduction to 20% of energy and increased fruits, vegetables and grain intake, or a usual diet control group (60%, n=29,294). As previously reported, the dietary modification program reduced fat intake, increased fruit, vegetable and grain intake and was associated with modest weight loss (all P < 0.001). The current secondary analysis outcome is breast cancer overall survival in subgroups defined by breast cancer characteristics for cases diagnosed during the dietary intervention period. Because of possible selection prior to breast cancer diagnosis, these analyses do not compare randomized outcomes. Therefore, careful attention is paid to control of risk factors for breast cancer in the analysis.

Results: The examined subgroups included histology (ductal, lobular, other), estrogen receptor (ER) status (positive vs. negative by local laboratory), progesterone receptor (PR) status, HER2 status, triple negative (yes/no), stage (local, regional or distant), grade (well, moderately, poorly differentiated), tumor size (<1, 1- 2, >=2 cm), and nodal involvement (none, 1-3, 4+). None of the tests of interaction in subgroups were statistically significant. All subgroup hazard ratios (HR) were less than one except for ER negative cancers, triple negative cancers and those with 4+ positive lymph nodes. The results are suggestive of no influence of the low-fat dietary pattern on triple negative cancers (HR 1.64 95% CI 0.73-3.70 for triple negative vs. HR 0.73 95% CI 0.56-0.95 for other breast cancers, interaction P=0.06).

Conclusion: Compared to a usual diet control group, women randomized to a dietary intervention group providing a low-fat dietary pattern had a significantly increased overall survival following a breast cancer diagnosis with the possible exception of those developing triple negative cancers.

2016 San Antonio Breast Cancer Symposium

Publication Number: S5-05

Title: Impact of pre-operative exercise on breast cancer gene expression

Ligibel JA A, Irwin M, Dillon D, Barry W, Giobbie-Hurder A, Frank E, Winer EP P, McTiernan A, Cornwell M, Pun M, Brown M and Jeselsohn R. Dana-Farber Cancer Institute; Yale University; Brigham and Women's Hospital and Fred Hutchinson Cancer Research Center.

Body: Background: Exercise is linked to a lower risk of developing and dying from breast cancer, but the biological mechanisms through which exercise could impact breast cancer are unclear. In animal models, exercise impacts tumor formation and progression, but there are few data regarding direct effects of exercise on tumor tissue in humans. The Pre-Operative Health and Body (PreHAB) Study was a randomized window of opportunity trial designed to explore the impact of exercise on molecular pathways in women with breast cancer.

Methods: Inactive women with Stage I-III breast cancer were enrolled through Dana-Farber Cancer Institute and Yale University prior to surgery. Participants were randomized 1:1 to an aerobic and strength training exercise intervention or mind body control intervention and participated in the interventions between enrollment and the time of surgery. Tumor tissue was collected at enrollment and surgery; samples were reviewed by a breast pathologist and were macrodissected to include sections of tumor with at least 10% cellularity. Capture RNA-sequencing of the transcriptome coding regions was performed using the Illumina Truseq RNA access platform.

Results: 49 women were randomized (27 exercise and 22 control). At baseline, mean age was 52.6, BMI was 30.2kg/m² and exercise was 49 min/wk. Mean time between enrollment and surgery was 4.2 weeks. Participants in the exercise arm significantly increased exercise vs. controls (increase of 203 vs. 23 min/wk, $p < 0.0001$). Transcriptomic analysis was performed on the tumors from the pre and post intervention biopsies from 32 patients (16 exercise and 16 control). Quality Control analysis of the RNA-sequencing data showed an average read depth of 25 million reads per sample, mapping ~79% to exonic regions. Principal Component Analysis revealed no read bias or batch effects and unsupervised clustering showed that pre- and post-operative samples clustered together by patient. Differential gene expression analysis by DESeq2 revealed a limited number of individual genes with significant changes after the intervention. KEGG pathway analysis, however, of 214 KEGG pathways using the bioconductor package GAGE (Generally Applicable Gene-Set Enrichment for Pathway Analysis) demonstrated upregulation of 13 unique pathways between the baseline biopsy and surgical excision in exercise participants and none in mind body participants ($q < 0.1$). The top ranked upregulated pathway was cytokine-cytokine receptor interactions ($q = 6.93E-05$, set size=238 genes). IL6, CCL3 and other cytokines are among the genes upregulated in this pathway. Analysis also demonstrated downregulation of 13 unique pathways ($q < 0.1$) including cell cycle, RNA transport and DNA replication pathways, in exercise participants over the intervention period.

Conclusions: A pre-operative exercise intervention led to alterations in gene expression in tumor tissue in women with breast cancer. Validation in additional data sets and an analysis of which cellular compartments within the tumor are responsible for the changes is needed. These findings demonstrate that exercise may have a direct effect on breast tumor tissue in humans, providing new insights into the biologic mechanisms through which exercise could lower the risk of developing and dying from breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: S5-06

Title: Randomized, placebo-controlled trial of duloxetine for aromatase inhibitor (AI)-associated musculoskeletal symptoms (AIMSS) in early stage breast cancer (SWOG S1202)

Henry NL, Unger JM M, Schott AF F, Fehrenbacher L, Flynn PJ J, Prow D, Sharer CW W, Lew DL L, Moseley A, Fisch MJ J, Moinpour C, Hershman DL L and Wade III JL L. University of Michigan; Kaiser Permanente, Northern California; Metro Minnesota CCOP/Minnesota Oncology; William R. Bliss Cancer Center; Phoenixville Cancer Center; AIM Specialty Health; Fred Hutchinson Cancer Research Center; Heartland NCORP and Columbia University.

Body: Background: Adherence to AI therapy for adjuvant treatment of hormone receptor-positive breast cancer is poor, primarily because of AIMSS. Premature discontinuation of AI therapy can lead to increased likelihood of breast cancer recurrence. Duloxetine (dulox) is a serotonin norepinephrine reuptake inhibitor that is FDA-approved for treatment of multiple chronic pain disorders. Phase II data from an open label trial of dulox for treatment of AIMSS demonstrated a 61% improvement in pain. We hypothesized that treatment of AIMSS with dulox would improve average joint pain compared to placebo (plac).

Methods: Postmenopausal women with stage I-III breast cancer who had been taking AI therapy for between 3 wks and 36 mo were enrolled. To be eligible, patients were required to have average pain of $\geq 4/10$ using the Brief Pain Inventory (BPI) that developed or worsened since AI therapy initiation, and not have any contraindications to dulox therapy. Patients were randomized 1:1 to dulox 30 mg daily for 7 d then 60 mg daily for 11 wks then 30 mg daily for 7 d, or to matching plac, stratified by baseline pain (4-6 vs 7-10) and prior taxane use (yes vs no). Pain, depression, and quality of life (QoL) were assessed after 2, 6, and 12 wks of therapy, as well as at the 24 wk time point. The primary analysis used linear mixed models to examine average pain through 12 wks by arm, adjusting for the stratification factors and assessment time. Clinically significant change in average pain was defined as a ≥ 2 -point decrease from baseline.

Results: 299 patients were randomized between June 2013 and October 2015, 10 of whom were ineligible. 127 dulox-treated and 128 plac-treated patients were evaluable for the primary analysis. No sizeable imbalances in baseline factors were noted by arm. Seventeen pts reported grade 3 adverse events (AEs) (dulox: 12/138 (8.7%), plac: 5/141 (3.5%)), and 40 pts discontinued treatment because of AEs (dulox: 21 (52.5%), plac: 19 (47.5%)). Mean observed average pain, the proportion of pts experiencing clinically significant change in average pain from baseline, and percent reduction in average pain all indicated greater improvement for dulox compared with plac through 12 wks, but were similar by arm at wk 24 (12 wks after completion of intervention; see Table). In multivariable linear mixed model analysis, the BPI average pain was reduced on average by 0.82 points more on dulox compared to plac over the first 12 wks (95% CI -1.24 to -0.40, $p=0.0002$). Similar patterns were observed for worst pain, pain interference, joint pain, stiffness, and functioning, and QoL.

Table: Observed Average Pain Scores by Assessment Time										
	Time Point									
	Baseline		2 weeks		6 weeks		12 weeks		24 weeks	
	dulox	plac	dulox	plac	dulox	plac	dulox	plac	dulox	plac
Average pain	5.44	5.49	3.51	4.41	2.95	3.96	2.91	3.45	3.37	3.42
Percent reduction	-	-	34%	20%	46%	28%	46%	36%	37%	37%
Patients with clinically significant change	-	-	54%	44%	69%	49%	69%	60%	60%	59%

Conclusions: Treatment with duloxetine was superior to placebo for the treatment of AIMSS among women with early stage breast cancer, was well tolerated, and was associated with improvements in QoL.
Clinicaltrials.gov NCT01598298.

2016 San Antonio Breast Cancer Symposium

Publication Number: S5-07

Title: Aromatase inhibitors and endothelial function: Is there an association with early cardiovascular disease?

Blaes AH H, Beckwith H, Hebbel R, Solovey A, Potter D, Yee D, Vogel R, Luepker R and Duprez D. University of Minnesota, Minneapolis, MN.

Body: Background: As more women are cured from their breast cancer, survivors with early stage breast cancer are at greater risk of dying from cardiovascular disease than their breast cancer. Aromatase inhibitors (AI) have been shown to reduce breast cancer-related mortality in women with estrogen receptor (ER)-positive disease which makes up 75% of all breast cancer cases. The use of AIs has been associated with higher rates of hypertension, hypercholesterolemia, angina pectoris and ischemic cardiovascular disease. In the aging population taking AIs, little is known about the direct impact of AIs on endothelial function, a predictor of cardiovascular disease. Endothelial dysfunction identified by reactive hyperemia using Endo-PAT has been associated with an increased risk of cardiac adverse events, independent of Framingham risk score.

Methods: At the University of Minnesota in 2014-2015, 25 healthy postmenopausal women and 36 postmenopausal women with locally advanced breast cancer and prescribed an aromatase inhibitor were identified. Subjects with a history of hypertension or hyperlipidemia were excluded. Consented subjects underwent biomarker analysis and pulse wave analysis using the HDI/Pulse Wave CR-2000 Cardiovascular Profiling System and pulse contour analysis using the Endo-PAT2000 system. Biomarkers and functional test markers were compared between cases and controls using T-tests and Wilcoxon Rank-Sum tests.

Results: Mean age (61.7 vs 58.8 years), body mass index (27.4 vs 26.2 kg/m²), race (93% vs 92% Caucasian), and tobacco use (100% nonsmokers) were similar between cases and controls, respectively. Mean systolic blood pressure (BP) was elevated in cases (128.3 mmHg vs 114.5 mmHg, p=0.0006). There were no differences in lipid profiles. Median ultrasensitive estradiol levels were reduced in cases (2 vs 15 pg/mL, p<0.0001). Median high sensitive C-reactive protein was significantly elevated in cases (4146 vs 1406 ng/L, p=0.05). There were no differences seen in markers of hemostasis or endothelial damage, including circulating endothelial cells, vascular cell adhesion molecule, P-selectin. Median large artery elasticity (12.5 vs 15.1 ml/mmHg, p=0.02), small artery elasticity (5.2 vs 6.7 ml/mmHg, p=0.04), and endoPAT ratio (0.8 vs 2.6, p<0.0001) were significantly reduced in breast cancer survivors on AIs as compared to controls. There was no correlation between use of chemotherapy, radiation therapy, type of AI, or duration of AI use and endothelial function among the cases. When adjusting for differences in BP, endoPAT ratio continued to remain significantly decreased in breast cancer survivors (0.8 vs 2.6, p<0.0001).

Conclusion: Postmenopausal women with breast cancer on AIs have reductions in endothelial function, a predictor of adverse cardiovascular disease (acute coronary syndrome, chest pain, myocardial infarction, cardiac death). With the growing trend that longer duration of endocrine therapy is needed, further work is needed to confirm these findings.

2016 San Antonio Breast Cancer Symposium

Publication Number: S6-01

Title: *BRCA1* methylation status, silencing and treatment effect in the TNT trial: A randomized phase III trial of carboplatin compared with docetaxel for patients with metastatic or recurrent locally advanced triple negative or *BRCA1/2* breast cancer (CRUK/07/012)

Tutt A, Cheang MCU, Kilburn L, Tovey H, Gillett C, Pinder S, Lanchbury J, Abraham J, Barrett S, Barrett-Lee P, Chan S, Gazinska P, Grigoriadis A, Kernaghan S, Hoadley K, Gutin A, Harper-Wynne C, Hatton M, Owen J, Parker P, Roylance R, Shaw A, Smith I, Thompson R, Timms K, Wardley A, Wilson G, Harries M, Ellis P, Ashworth A, Perou C, Bliss J, Rahman N, Brown R and On Behalf of the TNT Trial Management Group and Investigators. Institute of Cancer Research, Breast Cancer Now, London, United Kingdom; Institute of Cancer Research Clinical Trials & Statistics Unit (ICR-CTSU), London, United Kingdom; King's College London School of Medicine, London, United Kingdom; Myriad Genetics, Inc, Salt Lake City, UT; Velindre NHS Trust Cancer Centre, Cardiff, United Kingdom; The Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom; Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; Breast Cancer No2 Unit, Guys & St Thomas NHS Trust, Kings College London; Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, Chapel Hill, NC; Maidstone and Tunbridge Wells NHS Trust, Maidstone, United Kingdom; Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom; King's College London School of Medicine and Francis Crick Institute, London, United Kingdom; University College London Hospitals NHS Foundation Trust, London, United Kingdom; Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom; The Royal Marsden NHS Foundation Trust, London, United Kingdom; BME Cancer Communities, Nottingham, United Kingdom; The Christie NHS Foundation Trust, Manchester, United Kingdom; UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; The Institute of Cancer Research, London, United Kingdom and Imperial College London, London, United Kingdom.

Body: Background

TNT previously reported that patients(pts) with advanced triple negative breast cancer(TNBC) and germline *BRCA1/2*(g*BRCA+*) mutations are more likely to respond to carboplatin(C) than to docetaxel(D), and have longer progression-free survival with C. Here, we report results from a pre-planned biological analysis to test whether *BRCA* silencing or methylation status confer sensitivity to platinum.

Methods

Pts eligible for TNT had ER-, PgR-, HER2- BC or were known g*BRCA+*(any ER/PgR/HER2). Pts were randomized to C(AUC 6 q3wk) or D(100mg/m² q3wk) for 6-8 cycles or until disease progression if sooner, with crossover possible on progression. Blood and archival tissue samples were requested for *BRCA1/2* genotyping, central analysis of TN and DNA repair deficiency biomarkers. Primary endpoint was intention-to-treat objective response rate(ORR) up to cycle 6. *BRCA1* mRNA level was estimated from whole-genome total RNA sequencing(RNA-Seq). *BRCA1* methylation analysis was quantified by bisulfite sequencing. A methylation score for the sample was computed as percentage of methylated reads relative to total number of reads that were either methylated or not methylated for CpG sites. A sample with a score >10% was considered methylated(*BRCA1*^{meth})^{3/4} a *BRCA1* mRNA level "silenced" subgroup(*BRCA1* silencing) was defined as value of log₂(mRNA)<8.4, based on bimodal distribution.

Results

212 and 191 pts' samples were assessed for *BRCA1* methylation and mRNA levels. 33 pts (15.6%) were found to be *BRCA1*^{meth}. Pts with *BRCA1*^{meth} had a better response to D (42%, 95%CI: 20, 64) than C (21%, 95%CI: -0.1, 43)^{1/4} absolute difference (C-D) -21% (95%CI: -52, 10)^{1/4} p=0.28), but not statistically significant. There was no evidence of a difference when g*BRCA+* tumours were excluded.

31 pts (16%) had *BRCA1* silencing. Concordant with *BRCA1*^{meth}, pts with *BRCA1* silencing had a better response to D (65%, 95%CI: 42, 87) than C (29%, 95%CI: 4.9, 52)^{1/4} absolute difference (C-D) -36% (95%CI: -69, -3.3)^{1/4} p=0.07), with an odds ratio 0.22 (95%CI: 0.035, 1.2) in favor of D, interaction with treatment not significant (p=0.066).

19 pts with *BRCA1*^{meth} and *BRCA1* silencing had a better response to D (60%, 95%CI: 30, 90) than C (22%, 95%CI: -5.0, 49)^{1/4} absolute difference (C-D) -38% (95%CI: -79, 2.9) in favor of D (p=0.17), and the interaction was not significant (p=0.15).

Further exploratory analyses examining any relationship between a response to C and new cutpoints for *BRCA1*^{meth} or *BRCA1* mRNA level did not suggest any evidence of a signal.

Conclusion

Our data did not support a priori hypotheses that *BRCA1* methylation or silencing would be associated with a greater response to C than D, which is consistent with other reports in ovarian cancer. As *BRCA1* methylation will not always lead to significant silencing or be the only cause of low *BRCA1* gene expression, exploratory analysis is investigating the interaction of BRCA silenced group with D sensitivity. RNA-seq is ongoing and results from the final database will be presented.

2016 San Antonio Breast Cancer Symposium

Publication Number: S6-02

Title: The efficacy and safety of the addition of ibandronate to adjuvant hormonal therapy in postmenopausal women with hormone-receptor positive early breast cancer. First results of the TEAM IIB trial (BOOG 2006-04)

Vliek SB B, Meershoek-Klein Kranenbarg E, van Rossum AGJ GJ, Tanis BC C, Putter H, van der Velden AWG WG, Hendriks MP P, van Bochove A, van Riet Y, van Leeuwen-Stok AE E, Tjan-Heijnen VCG CG, Kroep JR R, Nortier JWR WR, van de Velde CJH JH and Linn SC C. Netherlands Cancer Institute, Amsterdam, Netherlands; Leiden University Medical Center, Leiden, Netherlands; Maastricht University Medical Center, Maastricht, Netherlands; Martini Ziekenhuis, Groningen, Netherlands; Northwest Clinics, Alkmaar, Netherlands; Zaans Medisch Centrum, Zaandam, Netherlands; Catharina Ziekenhuis, Eindhoven, Netherlands; Groene Hart Ziekenhuis, Gouda, Netherlands and Dutch Breast Cancer Research Group, Amsterdam, Netherlands.

Body: Background:

Results of clinical trials concerning adjuvant bisphosphonates for the prevention of (bone) metastases in patients with early breast cancer are conflicting. A recent large meta-analysis, however, suggests that bisphosphonates reduce the incidence of (bone) metastases and improve skeletal-related events in early breast cancer patients. Subgroup analyses show that postmenopausal women seem to benefit the most. In this subgroup a modest overall survival benefit was observed with the addition of adjuvant bisphosphonates to standard adjuvant systemic therapy (EBCTCG, Lancet, 2015). TEAM IIB, a randomized phase III study (ISRCTN17633610), prospectively investigates the value of the addition of ibandronate to adjuvant hormonal therapy in postmenopausal women with hormone receptor-positive breast cancer.

Methods:

Postmenopausal women with stage I-III breast cancer and an indication for adjuvant hormonal treatment were randomized to receive at least 5 years of hormonal therapy (tamoxifen followed by at least 2-3 years exemestane, or in case of high risk at least 5 years of exemestane) with or without ibandronate 50mg orally, once daily for three years. Primary endpoint was disease-free survival (DFS). Secondary endpoints included time to and rate of bone metastases, other sites of recurrence, overall survival and safety. The study was amended because of slower than anticipated accrual and the sample size calculations were amended accordingly in June 2009. To detect a hazard ratio (HR) of 0.615 with a 2-sided alpha of 0.05 and a power of 0.8, 139 DFS-events were required in the intention-to-treat population.

Results: Between February 2007 and May 2014, 1116 patients were enrolled in 37 hospitals in the Netherlands of whom 40% had positive axillary lymph nodes and 56% of all patients received (neo)adjuvant chemotherapy (>95% anthracyclines, 69% taxanes). Baseline characteristics were well balanced. At September 9, 2016, 143 DFS events had been reported. Median follow-up was 4.6 years and 80 patients were still on ibandronate treatment. Adherence to 3 years ibandronate was 67%, 21 patients randomized to receive ibandronate never started. 19 patients, of whom 9 in the control group were excluded because of major ineligibility.

In the ibandronate treated group 3-year DFS was 94.4% versus 90.8% in the control group (HR 0.84; 95% confidence interval [CI] 0.60-1.17). In total, 48 patients in the ibandronate versus 45 in the control group died, of whom 18 (37,5%) versus 28 (62,2%) of breast cancer. 3 years after randomization 1.6% of ibandronate treated patients developed bone metastases versus 4.6% in patients who were treated with adjuvant hormonal therapy only (HR 0.76; [CI] 0.43-1.32). 14 (29,2%) versus 9 (20%) of patients died because of secondary malignancies respectively.

There was no significant difference in creatinine clearance during the first three years after randomization. 36 Serious adverse events (SAEs) were reported in the ibandronate group versus 51 in the control group. Of patients randomized to ibandronate 4 developed osteonecrosis, but without residual complaints.

Conclusion: So far, at a median follow-up of 4.6 years there is no statistically significant benefit from adding ibandronate to adjuvant hormonal treatment in postmenopausal women with hormone-receptor positive early breast cancer. However, since hazard rates are in favor of ibandronate longer follow-up is warranted before final conclusions can be drawn.

2016 San Antonio Breast Cancer Symposium

Publication Number: S6-03

Title: DBCG 07-READ: A randomized phase III trial comparing six cycles of docetaxel and cyclophosphamide (DC) to three cycles of epirubicin and cyclophosphamide followed by three cycles of docetaxel (EC-D) in patients with early breast cancer

Ejlertsen B, Tuxen MK K, Jakobsen EH H, Jensen M-B, Knoop AS S, Hoejris I, Ewertz M, Balslev E, Vestlev PM, Kenholm J, Nielsen DL L, Bechmann T, Andersson M, Cold S, Nielsen HM M, Maae E, Carlsen D and Mouridsen HT T. Danish Breast Cancer Cooperative Group, Copenhagen, Denmark.

Body: Background: DBCG 07-READ was designed to compare sequential EC followed by D with DC in patients with early, TOP2A normal breast cancer as a retrospective evaluation of the DBCG 89D trial suggested that these patients would not benefit from an anthracycline.

Methods: This is a multicenter open-label randomized phase III trial. Three groups of women were eligible following completely resected unilateral invasive TOP2A normal (TOP2A gene to centromere 17 ratio of 0.8 to 2.0) breast cancer by mastectomy or breast conserving surgery in combination with axillary clearance or a negative sentinel node biopsy; 1: Age 18 to 39 years; 2: Age 40 to 75 years and estrogen receptor (ER) negative (<10% positive) and/or HER2 positive tumor; and 3: Age 40 to 59 years and ER \geq 10% positive and either node positive, ductal carcinoma and grade II-III, or tumor size > 20 mm. Eligible patients were required to have a Charlson Comorbidity (CC) Index \leq 2 and to be without signs of distant metastasis. Patients were randomized to receive 6 cycles of DC (docetaxel 75 mg/m² and cyclophosphamide 600 mg/m²) every 3 weeks or 3 cycles of EC (epirubicin 90 mg/m² and cyclophosphamide 600 mg/m²) followed by 3 cycles of D (docetaxel 100 mg/m²) every 3 weeks. In case of a CC Index of 1 or 2 chemotherapy was administered at a reduced dose-intensity. Adjuvant endocrine treatment, trastuzumab and radiotherapy were administered according to the guidelines of the DBCG. The primary endpoint was disease-free survival (DFS), and secondary endpoints were overall survival (OS) and distant disease-free survival (DDFS).

Results: Between July 2008 and December 2012 we (12 DBCG centers) randomly assigned 2006 eligible patients to DC (N=1008) or EC-D (N=998). Patient and tumor characteristics were balanced by treatment groups. The median estimated potential follow-up is 5.4 years and the 5-year DFS was 88.0%; 95% CI 85.8 to 90.0 in the EC-D arm and 87.9%; 95% CI 85.7 to 89.9 in the DC arm. No significant difference in the risk of DFS events HR=1.03; 95% CI 0.80 to 1.32; p=0.84 or mortality HR=1.11; 95% 0.79 to 1.56; p=0.55 was observed in the intent to treat analysis. Patient-reported toxicity will be compared for the two chemotherapy regimens.

Conclusion: The READ trial gives evidence to support no outcome benefit from anthracycline in patients with TOP2A normal early breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: S6-04

Title: The PI3K inhibitor, taselisib, has enhanced potency in PIK3CA mutant models through a unique mechanism of action

Friedman LS S, Edgar KA A, Song K, Schmidt S, Kirkpatrick DS S, Phu L, Nannini MA A, Hong R, Cheng E, Crocker L, Young A and Sampath D. Genentech, Inc, South San Francisco, CA.

Body: Alterations of the phosphoinositide-3 kinase (PI3K)/Akt signaling pathway occur broadly in cancer via multiple mechanisms including mutational activation of the *PIK3CA* gene. The dysregulation of this pathway has been implicated in tumor cell growth and survival, thus PI3K is a promising therapeutic target with multiple inhibitors in clinical trials. Taselisib (GDC-0032), a novel, oral, selective inhibitor of p110alpha, sparing inhibition of p110beta, is more potent against cancer cells bearing mutations in the *PIK3CA* gene than those with wildtype *PIK3CA*. The mechanism leading to this enhanced mutant selectivity is revealed in these preclinical studies.

Uniquely among PI3K inhibitors, taselisib has a gain of potency in *PIK3CA* mutant SW48 isogenic cells compared to wildtype SW48 parental cells. Pathway inhibition and increased apoptosis are associated with the enhanced activity observed in *PIK3CA* mutant cells. In *PIK3CA* mutant cell culture-derived and patient-derived xenograft (PDX) models taselisib induces tumor regressions. In comparison to other clinical-stage PI3K inhibitors, taselisib confers superior anti-tumor activity in *PIK3CA* mutant xenografts when treated at a Maximum Tolerated Dose (MTD) in vivo.

We have discovered that taselisib has a dual mechanism of action, both blocking kinase signaling and inducing down-regulation of the mutant p110alpha protein level in a dose-dependent and time-dependent manner. Taselisib treatment leads to the specific degradation of mutant p110alpha without significant change in wildtype p110alpha protein in cultured cells and in mutant xenograft models including PDX. Other clinical PI3K inhibitors, including PI3Kalpha selective and pan-PI3K inhibitors are unable to induce degradation of mutant p110 alpha.

The taselisib-induced degradation of mutant p110a protein is ubiquitin-mediated and proteasome-dependent. These unique mechanistic effects of taselisib are most pronounced when comparing signaling suppression and p110a protein levels at 24 hours vs. 1 hour of drug exposure in *PIK3CA* mutant cell lines. This discovery indicates that PI3K inhibitors which trigger degradation of mutant p110a protein can more effectively suppress the signaling pathway in response to feedback, and may result in greater activity and improved therapeutic index.

2016 San Antonio Breast Cancer Symposium

Publication Number: S6-05

Title: Comprehensive comparison of prognostic signatures for breast cancer in TransATAC

Sestak I, Buus R, Cuzick J, Dubsy P, Kronenwett R, Ferree S, Sgroi D, Schnabel C, Baehner R, Mallon E and Dowsett M. Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, QMUL, London, United Kingdom; Institute of Cancer Research, London, United Kingdom; BrustZentrum Klinik St. Anna, Lucerne, Switzerland; Sividon Diagnostics, Cologne, Germany; NanoString Technologies, Seattle, WA; Massachusetts General Hospital, Boston, MA; bioTheranostics, San Diego, CA; Genomic Health, Redwood City, CA and NHS Greater Glasgow and Clyde, Glasgow, United Kingdom.

Body: Background: A number of prognostic signatures have been developed for the prediction of breast cancer recurrence in the past decade. We have developed two signatures (Clinical Treatment Score (CTS), four immunohistochemical markers (IHC4)) and validated four prognostic signatures (Oncotype Dx Recurrence Score (RS), PAM50-based Prosigna (ROR), Breast Cancer Index (BCI), and EndoPredict (EPclin)) in the TransATAC cohort. Here, we compare the prognostic performance of these six signatures for distant recurrence (DR) in years 0-10, and specifically in years 5-10 after treatment cessation.

Methods: 1231 postmenopausal women with hormone receptor positive and HER2-negative breast cancer had at least one test performed. Of these, 818 women had data on all six signatures available. IHC4, RS and BCI (linear) are molecular only signatures whereas CTS, ROR and EPclin include clinicopathological factors. The primary endpoint was DR and the primary objective was to compare the prognostic value of the six signatures in terms of DR for years 0-10, 0-5, and 5-10. Secondary objectives included the comparison of the prognostic performance for node-negative and node-positive patients separately and the additional prognostic performance of each signature to the others. Likelihood ratio statistics ($LR-\chi^2$) were used to assess the prognostic information of each signature alone or in combination with other signatures.

Results: Median follow-up for this analysis was 9.94 years (IQR 8.01-10.09) and a total of 126 DR were recorded. 818 women with HER2-negative disease for whom data of all six signatures were available were included in this analysis. For all patients, CTS and EPclin were the most prognostic signatures in years 0-10 (CTS: $LR-\chi^2=124.9$; EPclin: $LR-\chi^2=116.2$) and years 5-10 (CTS: $LR-\chi^2=59.6$; EPclin: $LR-\chi^2=56.8$) in the univariate analysis. The other four signatures performed similarly well in years 0-5, but of those only BCI and ROR provided substantial prognostic information in years 5-10 (BCI: $LR-\chi^2=25.3$; ROR: $LR-\chi^2=43.8$). In multivariate analyses comparing the added information of the molecular signatures over CTS, IHC4 and BCI provided the most information (IHC4: $\Delta LR-\chi^2=19.0$; BCI: $\Delta LR-\chi^2=19.8$). In node-negative patients (72.3%), the ROR showed the most prognostic value in years 0-10 ($LR-\chi^2=48.6$) and years 5-10 ($LR-\chi^2=31.3$) whereas the RS was least prognostic in this patient group. For patients with node-positive disease (27.7%), the CTS and EPclin were the most prognostic and the other four signatures provided much less prognostic information for this patient population (data not shown).

Conclusion: Overall, the CTS and EPclin were the most prognostic signatures for DR and also added significant prognostic value to the other scores in women with HER2-negative disease, primarily due to the incorporation of nodal status in these signatures. For women with node-negative disease, the ROR, BCI, and EPclin signatures provided most prognostic value whereas for those with positive nodes CTS and EPclin were most prognostic. Our analyses showed that the inclusion of clinic-pathological factors into gene signatures is highly important for deriving an accurate prognostic assessment, particularly in node-positive patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: S6-07

Title: Double blinded validation study to assess performance of IBM artificial intelligence platform, Watson for oncology in comparison with Manipal multidisciplinary tumour board – First study of 638 breast cancer cases

Somashekhar SP, Kumarc R, Rauthan A, Arun KR, Patil P and Ramya YE. Manipal Comprehensive Cancer Centre, Manipal Hospital, Bangalore, Karnataka, India.

Body: Background:

IBM Watson for Oncology (WFO) developed in collaboration with Memorial Sloan Kettering Cancer Centre is a cognitive computing system able to extract structured data from free text documents using natural language processing (NLP). It is a technology platform that uses NLP and machine learning to reveal insights from large amounts of unstructured data. Currently WFO provides treatment options only for breast, lung and colorectal cancers. In the present study we try to evaluate concordance of WFO treatment recommendations with Manipal multidisciplinary tumor board (MMDT), a quaternary health care centre for 638 breast cancer cases.

Materials and Methods:

MMDT treatment recommendation and relevant clinic-pathological data of 638 breast cancer cases both localised (514) and metastatic (124) disease which were treated in last 3 years was collected and the collected data of all the patients was entered in WFO. The treatment recommendations & the time required to enter the data and the time taken by WFO to give recommendations after analyzing the data was recorded. Treatment recommendation by WFO came in three categories with colour coding green, orange and red. Green is the RECommended standard treatment (REC), Orange is For Consideration (FC) and Red is Not RECommended (NREC). Concordance between MMDT and WFO was analysed for all cases as per stage and receptor status.

Results:

Of the treatment recommendations given by MMDT, WFO provided 46.4% in REC, 26.1% in FC, 21.5% in NREC. Nearly 73% of the MMDT treatment recommendations were in WFO REC and WFO-FC group. However 6% of the treatment provided by MMDT was not available with WFO. Treatment recommendations from WFO were concordant with MMDT in nearly 80% of time in non-metastatic and 45% in metastatic disease. In subset analysis of breast cancer with respect to receptor status the WFO-REC treatment was high with triple negative disease with 67.9% and least with Her 2neu negative disease with 35%. Across all subsets with respect to receptor status concordance was better with non-metastatic disease over metastatic disease which was statistically significant. The mean time taken to collect the data and enter was 20 minutes which gradually decreased with acquaintance after 10 cases to 12 minutes. Metastatic disease took longer time (5-7 minutes more) over localized disease in all the groups. WFO took a median of 40 seconds to capture, analyze and give the treatment recommendations.

Conclusions:

WFO-REC and WFO-FC together were in 73 % of time concordant with the MMDT treatment recommendations. However with respect to metastatic disease and hormone positive Her 2 neu –ve disease there still needs to be lots of improvement from WFO. WFO is a step towards personalized medicine. It should be kept in mind that WFO will be only an assisting tool and it will never be able to replace the patient-doctor relationship which is a very essential component of treating a patient suffering with cancer. WFO will be a reliable artificial intelligence tool for every cancer center and its multidisciplinary tumor board and will change the quality of care in oncology.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-01-01

Title: A phase II study of PD-L1 and CTLA-4 inhibition and immunopharmacogenomics in metastatic breast cancer

Santa-Maria CA A, Jain S, Flaum L, Park J-H, Kato T, Gross L, Uthe R, Tellez C, Stein R, Rademaker A, Gradishar WJ J, Nakamura Y, Giles FJ J and Cristofanilli M. Northwestern University, Chicago, IL and University of Chicago, Chicago, IL.

Body: Background

A hallmark of cancer is its ability to evade the immune system, however, it can be harnessed to detect and destroy cancer cells through inhibition of immune checkpoints such as CTLA-4 and PD-L1. This strategy has complementary and non-redundant mechanisms resulting in immune activation and antitumor synergy; progression free survival benefit has already been demonstrated in melanoma. A critical barrier in developing immunotherapies, however, is the identification of predictive biomarkers of response to therapy. Immunopharmacogenomic biomarkers, such as mutational burden, neoantigen profiles, and T cell receptor sequencing will elucidate the molecular interface between cancer and immune system, and may predict those most likely to benefit.

Methods

A single arm Phase II study was designed to determine the efficacy of PD-L1 and CTLA-4 inhibition and effects on immunopharmacogenomic dynamics in patients with metastatic breast cancer. The primary endpoint of this proposal is to investigate the response rate of the PD-L1 inhibitor, durvalumab, and the CTLA-4 inhibitor, tremelimumab, in metastatic breast cancer; secondary endpoints will examine the T cell receptor repertoire clonality, tumor mutational burden and neoantigen profiles. A total of 30 patients will be enrolled and treated with durvalumab 1500mg IV and tremelimumab 75mg IV monthly for 4 doses, then durvalumab 750mg every 2 weeks for 18 doses to complete 1 year of therapy with the option to renew therapy for an additional year; biopsies and blood at baseline and 2 months will be collected to assess immunopharmacogenomic biomarkers. Patients are eligible if they have triple negative or ER-positive breast cancer and have progressed on at least one line of chemotherapy and standard endocrine therapy if applicable. This is the first study to investigate immunopharmacogenomic biomarkers of response to dual checkpoint blockade in patients with metastatic breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-01-02

Title: CNIO-BR-008 trial: Reversion of T-cell exhaustion caused by chronic treatment with hypoxia-inducing antiangiogenic treatment by durvalumab in HER2-negative breast cancer: A pilot proof-of-concept trial

Quintela-Fandino M, Manso L, Mouron S, Lopez-Acosta JF F, García-Saenz JA A, Holgado E, Pascual-Martinez T, Medina L, Guerra J, Gonzalez-Cortijo L and Mañes S. CNIO - Spanish National Cancer Research Center, Madrid, Spain; H12O -Hospital 12 de Octubre, Madrid, Spain; HSCC - Hospital Clinico San Carlos, Madrid, Spain; HRYC - Hospital Ramon y Cajal, Madrid, Spain; HLP - Hospital La Princesa, Madrid, Spain; HUF - Hospital Universitario Fuenlabrada, Madrid, Spain; HQ - Hospital Quiron, Madrid, Spain and CNB - Centro Nacional de Biotecnología, Madrid, Spain.

Body: Background: The role of immune checkpoints in tumor progression is less relevant in breast cancer than in other malignancies. However, in preclinical experimentation we found a niche for immune checkpoint inhibitors. In an immunocompetent model of HER2-negative breast cancer (MMTV-PyMT) we observed that antiangiogenic agents may induce either a tumor normoxic or hypoxic adaptation. Normoxic adaptation leads to a therapeutically exploitable dependence on mitochondrial metabolism (Cell Rep 2016; 15: 1-14). Hypoxic adaptation (usually caused by monoclonal antibody type antiangiogenics) induces an increase in PGE2 and PGA synthesis, followed by a switch of the tryptophan metabolism from 5HIAA to kinurenine that culminates in differentiation of naive T-cells to Tregs and expression of PD-L1 in the hypoxic areas. The exhausted T-cell response observed in this model can be restored with PD-L1 inhibitors. We sought to prove the relevance of T-cell exhaustion and its reversibility by the anti-PD-L1 antibody durvalumab in patients receiving chronic bevacizumab (Bev).

Trial design

Single-arm, prospective, multicentric, phase II open-label trial. Patients receiving Bev maintenance after a chemotherapy+Bev regimen administered in the first line of metastatic disease that experience disease progression (PD) during maintenance will be candidates. The treatment will start by adding durvalumab (10 mg/kg q2w) to the ongoing Bev (15 mg/kg q3 w). Patients will undergo serial tumor biopsies, tumor-cfDNA sequencing (baseline and progression), and immunophenotyping (baseline and q4w). RECIST/IR-RECIST and NCI CTC AE V4.03 criteria will be used for assessing disease response and toxicity.

Eligibility criteria

Women >18 year old diagnosed of HER2-negative advanced breast cancer; 2) have received chemotherapy plus Bev for the first line treatment and experienced PR, CR or SD, 3) followed by maintenance with three-weekly Bev in monotherapy for at least 6 weeks before diagnosis of PD. 4) Concurrent hormonal therapy is allowed for ER+ patients, but reception of previous immunotherapy is precluded. 5) Adequate organ function defined according to standard parameters.

Specific aims

Primary:

- 1) To determine the relative percentages of innate and adaptive immune cell subpopulations and ascertain the status of T-cell function and polarization by multiparametric flow cytometry in patients with acquired resistance against Bev
- 2) To assess the reversibility of the abnormalities evidenced in (1) by durvalumab
- 3) To determine the disease control rate of the combination, and its relationship with (1) and (2)

Secondary:

- 4) To determine potential tumor neoantigens generated by chronic tumor hypoxia secondary to the antiangiogenic treatment.

Statistical methods

The sample size is calculated on the basis of the expected change in the Treg percentage in peripheral blood (10%), with an alpha and beta errors of 5% and 20% respectively. The minimum number of patients necessary to observe a 10% decrease is 25. Changes in lymphocytes will be compared with intra-subject measurements and Z-scores.

Accrual: 2 of 25 (target accrual) patients have been recruited.

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2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-01-03

Title: A phase I trial of the safety and immunogenicity of a DNA plasmid based vaccine (WOKVAC) encoding epitopes derived from three breast cancer antigens (IGFBP2, HER2, and IGF1R) in patients with breast cancer

Childs JS S, Higgins DM M, DeShong K, Heckman-Stoddard BM M, Wojtowicz ME E, Stanton SE E, Bailey HH H, Wisinski KB B and Disis ML L. University of Washington, Seattle, WA; University of Wisconsin, Madison, WI and NCI, NIH, Bethesda, MD.

Body: Background: Three proteins insulin like growth factor binding protein 2 (IGFBP2), human epidermal growth factor receptor 2 (HER2), and insulin like growth factor receptor-1 (IGF1R) are overexpressed in pre-invasive and high risk breast lesions and are associated with progression to invasive breast cancer. These proteins are immunogenic and elicit both humoral and cellular immunity in breast cancer patients. It is hypothesized that immunization with a plasmid vaccine (WOKVAC) targeting antigens from these proteins will be safe and immunogenic. WOKVAC has been designed to include extended sequences of the immunizing antigens that are predominantly associated with eliciting Type I immune responses. Type I immunity results in immune cells called T-cells secreting high levels of inflammatory cytokines (called Th1) that stimulate tumor destruction as well as the generation of cytotoxic T-cells that can directly kill the tumors.

Trial design: Phase I dose escalation study of 3 doses of WOKVAC admixed with 100mcg of GM-CSF. Patients will be assigned sequentially to one of three arms (10 patients/arm): Arm 1=150mcg, Arm 2=300mcg, Arm 3=600mcg. Each dose arm will have a staggered enrollment to assess toxicity. If the Arm 1 dose is determined to be safe, Arm 2 patients can be enrolled. If the Arm 2 dose is safe and immunologically more efficacious than Arm 1 then Arm 3 patients can be enrolled. Study treatment includes 3 monthly vaccines, two evaluations at 1 and 6 months post vaccine and a 5 year follow-up to collect reports from the patient's primary oncologist. Toxicity is assessed at baseline through the end of the study. Serial blood draws for immunologic monitoring is done.

Eligibility criteria: Patients with non-metastatic, node positive, HER2 negative breast cancer that is in remission and defined as no evidence of disease. Patients must have a good performance status, be at least 28 days from last cytotoxic chemotherapy and/or radiotherapy and 28 days from any use of systemic steroids.

Specific aims: (1) Determine safety of 3 escalating doses of WOKVAC, (2) Determine the most immunogenic dose, (3) Determine whether a WOKVAC Th1 polyepitope plasmid based vaccine elicits a persistent memory T-cell and (4) Determine whether WOKVAC vaccination modulates T regulatory cells and myeloid derived suppressor cells.

Statistical methods: (1) Safety will be assessed per NCI CTCAE v. 4.0, (2) Immunogenicity will be defined by the magnitude of the Th1 IFN-gamma antigen specific immune response. Successful immunization is a protein specific IFN-g precursor frequency greater than 1:20,000 PBMC for each antigen or 2 fold increase if baseline immune response (3) The IGFBP2, HER2, and IGF1R specific IFN-g/IL-10 ratios by ELISPOT will be evaluated to determine that a predominantly Th1 immune response is stimulated, and (4) Humoral immune response will be measured by ELISA and serum antibody avidity for IGFBP2, HER2, and IGF1R to determine an avidity index (AI) before and after vaccination.

Targeted Accrual: 30 patients

Contact information:

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2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-01-04

Title: VADIS trial: Phase II trial of the nelipepimut-S peptide vaccine in women with DCIS of the breast

Mittendorf EA A, Plitas G, Garber J, Crew K, Heckman-Stoddard B, Wojtowicz M, Vornik L, Peoples GE E and Brown PH H. The University of Texas MD Anderson Cancer Center; Memorial Sloan Kettering Cancer Center; Dana Farber Cancer Institute; Columbia University; National Cancer Institute and Cancer Insight.

Body: Background: Our group has been investigating vaccination strategies in breast cancer. Specifically, we have been evaluating HER2-derived peptide vaccines including nelipepimut-S+GM-CSF administered adjuvantly to breast cancer patients who have been rendered disease-free with standard of care therapy but are at high risk for recurrence. Early phase clinical trials showed an approximately 50% reduction in relative recurrence risk in vaccinated patients. Based on these data, nelipepimut-S+GM-CSF is being evaluated in a phase III registration trial. Having shown the vaccine to be safe, effective in stimulating an antigen-specific immune response and potentially having clinical efficacy in the setting of secondary prevention, the current study was initiated to evaluate vaccination in DCIS patients. This trial represents an initial step to move the vaccine into the primary prevention setting.

Trial Design: Phase II, randomized, single-blind study. Patients will be randomized 2:1 to receive vaccine or GM-CSF alone. After enrollment, patients will receive 3 inoculations administered every other week preoperatively followed by surgery then completion of the vaccination series (3 additional inoculations) in the adjuvant setting.

Eligibility: The trial will enroll pre- or post-menopausal women with a diagnosis of DCIS made by core biopsy. The area of radiographic abnormality must measure at least 1 cm. Because the vaccine is a MHC class I, CD8+ T cell-eliciting vaccine, it is HLA restricted, and patients must be HLA-A2+ to enroll. Participants must also have an ECOG performance status <2, adequate cardiac, kidney and liver function and be willing to comply with all study interventions and follow-up procedures.

Specific Aims: The trial's primary endpoint is to evaluate for nelipepimut-specific CD8+ T cells in the peripheral blood of vaccinated patients compared to patients receiving GM-CSF alone. Secondary endpoints include evaluating toxicity; determining the immune response in vivo by DTH, in vitro by evaluating for epitope spreading to other tumor antigens, and importantly in the tumor by assessing the degree of lymphocytic infiltration in surgically resected specimens. The extent of HER2 expression, Ki67 and cleaved caspase 3 in the resected specimen will also be assessed.

Statistical Methods: A total of 108 DCIS patients will be consented and screened for eligibility. 48 (45%) are expected to be HLA-A2 positive. These 48 patients will be randomized 2:1 to vaccine or GM-CSF alone groups. Accounting for 10% attrition rate and for an approximately 5% non-evaluable sample rate, we expect to have 40 evaluable patients, 27 in the vaccine group and 13 in the GM-CSF alone group, that have baseline, pre-surgery, and post-surgery measures of nelipepimut-S-specific CD8+ T cells. We will have 82% power to detect a significant increase in nelipepimut-S-specific CD8+ T cells in the vaccine group versus the GM-CSF alone group.

Contact Info: The study is accruing at four sites to include Columbia University, Dana Farber Cancer Institute, MD Anderson Cancer Center and Memorial Sloan Kettering Cancer Center. Additional information can be obtained from the overall study PI, Dr. Elizabeth Mittendorf (eamitten@mdanderson.org). NCT0236582.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-01-05

Title: Functional MRI signatures of immune response to targeted breast cancer therapy

Gadi VK, Stanton S, Dintzis SM M, Calhoun KE E, Hippe DS S and Partridge SC C. University of Washington, Seattle, WA and Fred Hutchinson Cancer Research Center, Seattle, WA.

Body: Background: Emerging data has shown that the immune system participates in both tumor development and tumor elimination and control. In breast cancer, activation of the immune system may mediate the effects of several anticancer drugs. In HER2+ and triple negative breast cancers, even 10% increases in tumor infiltrating lymphocytes (TILs) predict improved prognosis to adjuvant chemotherapy. Furthermore, the monoclonal antibody trastuzumab is known to increase peripheral Th1 immunity in women with HER2+ breast cancer, which may be reflected by increased TILs. An imaging approach to measure and monitor immune response would provide an essential non-invasive tool to select appropriate therapies based on an individual's response. Dynamic contrast-enhanced (DCE) MRI, reflecting blood flow and capillary permeability, and diffusion weighted (DW) MRI, reflecting cellularity, hold unique potential to provide quantitative imaging markers of therapy-induced immune response in breast tumors.

Trial Design: We propose to use trastuzumab to invoke immune response in patients with HER2+ tumors. Each subject will undergo a multiparametric MRI exam (with DCE and DW-MRI) and tissue sampling before and after a single dose of targeted anti-HER2 therapy with trastuzumab, prior to surgery. Pretreatment tissue will be obtained from the diagnostic core biopsy, and post-trastuzumab tissue will be collected from a research biopsy or the surgical excision, requiring at most a single additional biopsy. A variety of quantitative parameters will be extracted from the MRI scans. Whole tumor characterization will be performed, to calculate histogram-based metrics and second order textural features. Functional MRI features will be compared with histologic assessment of TILs, VEGF expression, and other immune response markers in tumors treated with HER2-targeted therapy. After the study window, patients will undergo standard of care treatment at the discretion of their physician.

Specific Aims: The primary objective will be to identify quantitative MRI markers of immune response to HER2-targeted treatment. We will implement a high-resolution multiparametric MRI approach and determine whether early changes on MRI after a preoperative run-in dose of trastuzumab correlate with tumor immune response markers as measured by histologic assessment.

Statistical Methods: The magnitude of response will be determined for individual MRI and histologic markers following one cycle of trastuzumab, and Pearson and Spearman correlation coefficients will be used to assess associations between changes in MRI and histologic markers. With 50 patients, we will have 80% power to detect a significant correlation between MRI and histologic markers as small as $r = 0.39$ ($R^2 = 15\%$), based on a 2-sided test of the null hypothesis $r = 0$ at the 0.05 significance level.

Accrual: Three patients have been accrued to date, with a target of 50 HER2+ patients. Patients may consent to either 1) preoperative run-in of a single cycle of anti-HER2 treatment or 2) neoadjuvant targeted anti-HER2 only treatment regimen (e.g., co-enrollment in TBCRC 026 or comparable trial), either of which allows for monitoring at pre and post 1 cycle of anti-HER2 treatment.

Contact information: VK Gadi (vkgadi@uw.edu) or Savannah Partridge (scp3@uw.edu).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-01-06

Title: A phase I/IIa study of the whole-cell vaccine BriaVax™ in metastatic or locally recurrent breast cancer patients (NCT00095862)

Wiseman CL L and Lacher M. Briacell Therapeutics, Berkeley, CA.

Body: BACKGROUND: BriaVax™ (formerly SV-BR-GM-1) is a breast cancer cell line transfected to release GM-CSF. Under FDA BB-IND 10312, the vaccine was tested in 3 metastatic breast, 1 metastatic ovarian cancer patients refractory to previous therapy. Patients received 20 million viable cells ID at 4 sites 48-72 hours after low-dose cyclophosphamide, 300 mg/m². Mean cell count was 22.8 x10⁶ (18.8-27.6). Mean viability was 90.6%, (84-94%). Interferon-alpha (10,000 u) was injected into each inoculation site after 48 and 96 hrs. The protocol permitted 3 inoculations at 2 week intervals, then, if not showing clearly progressive disease, 3 more inoculations monthly. All patients were stable after 3 injections (2 months). However, Pt A002 enjoyed complete tumor regression of a progressing lung metastasis and near-complete response of multiple breast lesions (see below). Nonetheless, she relapsed widely 3 months after finishing the sixth and final injection per protocol. After obtaining FDA permission inoculations resumed. All metastases, including CNS, again showed prompt but subtotal regression after 3 immunizations (see Wiseman C and Kharazi A; The Breast Journal 2006). Toxicity was minimal and the overall survival of the 4 patients was 35 months.

TRIAL DESIGN: 9 patients will be accrued, toxicity (and also response) will be reviewed; unless there are prohibitive serious adverse events, 15 more patients will then be accrued.

ELIGIBILITY: Inclusion Criteria: Patients must have histological confirmation of breast cancer with recurrent and/or metastatic lesions via investigational site. Patients with new or progressive breast cancer metastatic to brain will be eligible if they meet other conditions. Patients must be 18 years of age or older, have expected survival of at least 4 months, adequate performance status (ECOG 0-2). Patients may be maintained on hormonal therapy provided there is clear evidence of tumor progression and have provided written informed consent.

Exclusion Criteria: Concurrent or recent chemotherapy (within 3 weeks), XRT within 3 weeks, may have had immunotherapy in the past (off within 3 weeks), or general anesthesia/major surgery (within 3 weeks). Patients must have recovered from all known or expected toxicities from previous treatment and passed a treatment-free "washout" period of 3 weeks before starting this program (8 weeks for persons receiving nitrosourea or mitomycin). History of clinical hypersensitivity to GM-CSF, interferon, yeast, beef, or to any components used in the preparation of the experimental vaccine. Additional criteria to be provided on request.

SPECIFIC AIMS: To evaluate the number, frequency, duration, and relation of toxicity events to BriaVax™ (formerly designated as SV-BR-1-GM), as defined by CTCAE and additional tests; to evaluate tumor response and durability; to assess immune responses to vaccine; to archive blood and urine for future analysis; to measure quality of life with the SF-36 questionnaire.

For further information, call the Briacell Corporate Office (US) at 888-485-6340

Acknowledgments: Dr. Gerhard Bauer, Dr. Saeid Babaei, the St. Vincent Medical Center, and Dr. George Peoples for advice and guidance.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-02-01

Title: POSITIVE: A study evaluating pregnancy and disease outcome and safety of interrupting endocrine therapy for young women with endocrine-responsive breast cancer who desire pregnancy (IBCSG 48-14/BIG 8-13)

Pagani O, Partridge AH H, Azim Jr. HA A, Peccatori F, Ruggeri M and Sun Z. International Breast Cancer Study Group, Bern, Switzerland.

Body: Background

Young breast cancer (BC) patients often face the disease before completing their family planning. The best available retrospective evidence suggests that pregnancy after BC does not negatively impact disease outcomes in patients with endocrine-responsive BC and is safe for offspring. However, given the possibility of prolonged adjuvant endocrine therapy (ET) (5-10 yrs), it is not feasible to wait until completion of therapy in many of these women and thus there is a need to explore the safety of temporary interruption of ET to allow pregnancy. To date, no prospective study has been conducted in young women attempting future pregnancy.

Trial Design

Young patients with endocrine-responsive early BC and pregnancy desire will interrupt ET for up to 2 yrs to attempt pregnancy. As resumption of menses and conception depends on many factors, e.g. patient's age and adjuvant treatment received, the 2-yr interruption period is approximate, intended to include treatment wash-out (3 mos) conception (~3-6 mos), delivery (~9 mos), breast feeding (~6 mos). Patients will be strongly advised to resume ET as soon as pregnancy attempts are concluded and to complete the planned 5-10 yrs ET.

Major Eligibility Criteria

- * Histologically-proven stage I-III endocrine-responsive BC.
- * Age ≥ 18 and ≤ 42 years at enrollment.
- * Adjuvant ET (SERM alone, GnRH analogue plus SERM or AI) for ≥ 18 mos but ≤ 30 mos, stopped within 1 mo prior to enrollment.
- * Patient wishes to become pregnant.
- * Premenopausal status at BC diagnosis.

Specific Aim

To assess the risk of BC relapse associated with temporary interruption of ET to permit pregnancy and to evaluate pregnancy success and offspring outcome.

Statistical Methods

A true risk of BC recurrence of 2% per year is assumed for patients who do not interrupt ET. With 500 patients enrolled in 4.0 yrs and an additional 1.6 yrs of follow up, there will be approximately 1600 patient-yrs of follow up and a median follow up of approximately 3 yrs at the time of the primary analysis, anticipated to occur 5.6 yrs after enrolment of the first patient. If the true risk of BC recurrence is 2% per yr, we anticipate 31 BC recurrences and an estimated 3-yr breast cancer free interval (BCFI) event of 5.6% (95% CI 4.0% to 7.9%).

Translational Research will investigate different ovarian function and uterine parameters; and circulating tumour DNA. FFPE tissue of the primary tumour will be collected to integrate different parameters related to biology of BC arising in young women. All material will be banked centrally.

Psycho-oncological Companion Study on fertility concerns, psychological well-being and decisional conflicts is mandatory in the US and open to interested centers elsewhere.

Accrual: Target: 500; Actual: 39 (30 Apr 2016)

Psycho-oncological Companion Study Accrual: Target: 200; Actual: 29 (30 Apr 2016)

Contact Information

POSITIVE is conducted and sponsored by the International Breast Cancer Study Group. The Alliance for Clinical Trials in Oncology is the US sponsor for NCTN network. Contact Monica Ruggeri, IBCSG Coordinating Center, monica.ruggeri@ibcsg.org, or Trial Coordinators at ibcsg48_positive@fstfrf.org.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-02-02

Title: ENDEAR: A randomized international phase 3 study comparing the efficacy and safety of enzalutamide in combination with paclitaxel chemotherapy or as monotherapy vs placebo with paclitaxel in patients with advanced diagnostic-positive triple-negative breast cancer

Dent R, Schmid P, Cortes J, Kim S-B, Andre F, Abramson V, Cardoso F, Colleoni M, Morris P, Steinberg J, Tudor IC, Uppal H, Paton VE E, Peterson A and Traina TA A. National Cancer Centre Singapore, Singapore; Brighton and Sussex Medical School, Brighton, United Kingdom; Vall D'Hebron Institute of Oncology, Barcelona, Spain; Ramon y Cajal University Hospital, Madrid, Spain; Asan Medical Center, Seoul, Korea; Institut Gustave Roussy, Villejuif, France; Vanderbilt-Ingram Cancer Center, Nashville, TN; Breast Unit, Champalimaud Clinical Centre, Lisbon, Portugal; Istituto Europeo di Oncologia, Milan, Italy; Memorial Sloan Kettering Cancer Center, New York, NY; Astellas Pharma, Inc., Northbrook, IL and Medivation Inc., San Francisco, CA.

Body: Background:Enzalutamide (ENZA), a potent androgen receptor (AR) inhibitor, demonstrated clinical activity in patients (pts) with advanced triple-negative breast cancer (aTNBC) (NCT01889238). TNBC with AR-driven biology has been previously confirmed; however, using immunohistochemistry (IHC) to identify this pt subset is suboptimal for AR-directed treatment. A novel binary genomic signature (Dx + or -) that identifies AR-driven disease was developed for its potential to select pts who may benefit from ENZA. Clinical outcomes in a phase 2 trial utilizing a genomic signature (Dx) showed improvement in Dx+ vs Dx- subgroups with ENZA being well tolerated; fatigue was the only grade ≥ 3 AE with a $\geq 5\%$ incidence (Table).¹

	Progression-Free Survival (mo) (Median, 95% CI)		Overall Survival (mo) (Median, 95% CI)	
	All	0-1 Prior Tx	All	0-1 Prior Tx
Dx+ (n=56)	3.7 (2.4-6.4)	7.5 (3.4-14)	21.3 (12.9-21.3)	NR (14-NR)
Dx- (n=62)	1.9 (1.6-2.9)	1.9 (1.6-3.7)	7.5 (4.8-11.2)	10 (6.6-NR)

Data cutoff date, Sep 15, 2015. NR=not reached; Tx=treatment.

Preclinically, ENZA + paclitaxel (PAC) has been shown in an MBA-MB-453 xenograft model to significantly reduce tumor volume compared to either single agent. Phase 1b dose-finding studies of ENZA + taxane combinations have shown the combination to be generally well tolerated.^{2,3} The aim of this phase 3 study is to evaluate and compare the clinical benefit and safety of ENZA + PAC or ENZA vs PAC alone in pts with Dx+ aTNBC.

Methods:ENDEAR is an international, double-blind, randomized, placebo-controlled, 3-arm phase 3 study in pts with Dx+ aTNBC who previously received 0-1 prior regimen for advanced disease. A total of 780 pts will be randomized (1:1:1) to 1 of 3 treatment arms: (A) ENZA + PAC; (B) placebo + PAC; (C) ENZA monotherapy followed by PAC at time of disease progression on ENZA monotherapy. Randomization will be stratified by the number of prior systemic therapies in the advanced disease setting (0 vs 1 line), prior taxane use in the neo/adjuvant setting (yes vs no), and geographic region of enrollment. Archival tissue samples are required for diagnostic assessment. Pts must have either measurable or evaluable disease; brain metastases and seizure history are exclusionary. PAC 90 mg/m² (dose reduction allowed) will be given intravenously weekly for ≥ 16 weeks; ENZA 160 mg/day or placebo will be given daily until progression or other discontinuation criteria are met (crossover prohibited). The primary endpoint is progression-free survival (PFS) as assessed by an independent review; the key secondary endpoint is overall survival (OS). Assessment of tumor response will be done every 8 weeks. A hierarchical testing plan will control for multiplicity across PFS and OS. A stratified log-rank test will be used to compare the treatment groups: arm A vs B and arm C vs B. The primary analysis will be performed when ≥ 360 PFS events occur in arms A and B. Recruitment is ongoing.

References:1. Medivation. Data on file.

2. Morris MJ et al. *Clin Cancer Res*. 2016 Feb 8 [Epub ahead of print].
3. Mahipal A et al. *J Clin Oncol*. 2015;33:e15250.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-02-03

Title: Long-term follow-up of TEXT and SOFT trials of adjuvant endocrine therapies for premenopausal women with HR+ early breast cancer

Francis PA A, Fleming GF F, Regan MM M, Pagani O, Walley BA A, Price KN N, Coates AS S, Goldhirsch A and Gelber R. International Breast Cancer Study Group (Switzerland), Breast International Group (Belgium), and North American Breast Cancer Group (USA).

Body: Background

First results of the TEXT and SOFT international phase III trials were practice-changing, indicating that: i) 5y adjuvant exemestane+ovarian function suppression (E+OFS) reduces recurrence risk relative to tamoxifen(T)+OFS or to T alone, ii) T+OFS reduces recurrence risk vs T in women who are at sufficient risk to warrant chemotherapy (CT) and remain premenopausal afterwards, and iii) T alone remains appropriate for some premenopausal women. However, median follow-up (FU) was only 5.5y and <5% pts had died. FU is immature given the long natural history of HR+ disease and EBCTCG overviews showing overall survival (OS) improvements for T vs no-T emerged during 5-15y. It is crucial to establish if changing standard adjuvant endocrine therapy from T improves survival and if there are associated late toxicities.

Trial Design and Aims

Premenopausal women had invasive early breast cancer (BC) assessed as $\geq 10\%$ ER and/or PgR.

SOFT was designed to determine the value of adding OFS to T, and the role of E+OFS in two cohorts: women who remained premenopausal after completion of neo/adjuvant CT, and women for whom adjuvant T alone was considered suitable treatment. SOFT compares 5y of T to T+OFS or E+OFS. OFS was GnRH analog triptorelin x5y, oophorectomy or ovarian irradiation. Median age was 43y; 35% had N+ disease. 53% enrolled after prior neo/adjuvant CT.

TEXT was designed to determine the role of adjuvant E in premenopausal women receiving OFS from the start of adjuvant therapy, comparing 5y of E+OFS vs T+OFS. Patients enrolled at start of all adjuvant therapy; 60% had CT concurrent with triptorelin after entry. Median age was 43y; 48% had N+ disease.

Secondary objectives include effects on OS, late side effects of early menopause and late toxicities.

Accruals

TEXT: 2672 women, Nov03-Mar11

SOFT: 3066 women, Dec03-Jan11

Statistical Methods

The primary endpoint, invasive disease-free survival, is time from randomization to invasive local, regional, or distant relapse, contralateral BC, second non-BC malignancy, or death. Secondary endpoints are BC-free interval, distant recurrence-free interval and OS. Primary results were reported in 2014, after ~5.5y median FU; 30% pts were still on 5y treatment and >90% continued in FU.

Long-term FU

Updated results are planned for FU through Dec16, with ~8y median FU. Pts finished 5y treatment by Apr16. Yearly visits continue; data collection includes weight, performance status, menstrual status, pregnancy attempts, GYN procedures, late AEs (cardiovascular, bone fracture), extended adjuvant therapy, invasive recurrence at first and subsequent sites, second non-BC malignancy, in situ cancers, OS.

FU through 2020 is planned, for min and median FU of 10 and 12y, roughly doubling the numbers of endpoints events since the first report. This will be critical to determine whether short-term treatment benefits persist for late recurrence, improve power to detect treatment effects on distant recurrence and OS endpoints with lower event rates occurring later in FU, and define associated late toxicities and side effects of early menopause. A consortium to fund long-term FU is being pursued.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-02-04

Title: A randomized controlled trial comparing acupuncture versus usual care for the treatment of aromatase inhibitor-induced arthralgia (AIIA) in women with early-stage breast cancer

Frank HS S, Bendinger GM M, Ensor Jr. JE E, Neufeld N, Nixon D, Randolph K, McGuire E, Rados K, McNight JE E, Pabbathi H, Panicker R, Johnson AT T, Lammersfeld C and Alvarez R. Cancer Treatment Centers of America, Newnan, GA and Houston Methodist Research Institute, Houston, TX.

Body: BACKGROUND: The increase in breast cancer (BC) survival is largely due to the benefits of hormonal therapy, such as tamoxifen and aromatase inhibitors (AIs), for the treatment of hormone-sensitive breast cancer. Recent clinical trials have demonstrated that AIs are more effective than tamoxifen at reducing BC recurrences. However, BC patients receiving AIs have a higher incidence of osteoporosis, bone fractures, and musculoskeletal symptoms, particularly joint pain and stiffness. The incidence of AIIA in the ATAC trial was 27.8% in patients taking anastrozole versus (vs) 21% in patients taking tamoxifen (Baum M, Lancet 2002). The arthralgia associated with AIs can be so debilitating that it contributes to a significant percentage of non-compliance and discontinuation of systemic treatment. Patients with cancer often show interest in complementary and integrative modalities for symptom management (Vickers AJ, Lancet 2001), of which Acupuncture (Ac) is one of the most utilized therapies.

ELIGIBILITY: Subjects must be female ≥ 18 years, have undergone mastectomy or breast-sparing surgery, and must have recovered from all pain-related effects of the surgery and radiotherapy with a minimum washout period of 30 days prior to registration. Patients should have ER and PR positive BC and be actively taking a 3rd-generation AI (Anastrozole, Letrozole, or Exemestane) for at least the 30 days prior to registration and plan to continue for at least one year after registration. ECOG PS ≤ 2 and platelets counts $\geq 50,000/\mu\text{L}$. Patients must not have received topical analgesic with exception of oral NSAIDs drugs ≤ 14 days to registration.

TRIAL DESIGN: This is a 12-week, two arm randomized (1:1) trial evaluating the benefit of Ac in BC patients experiencing AIIA.

AIMS: The aim of this study is to investigate the effectiveness of an integrative approach using Ac vs usual care (UC) treatment consisting of NSAIDs for the management of the AIIA. Primary endpoint is to compare the difference of Brief Pain Inventory-Short Form (BPI-SF) score at baseline vs week 8 between Ac and UC groups. Secondary endpoints are a) to compare the difference of BPI-SF score at baseline vs week 12 between Ac and UC groups, b) to compare the difference in AI adherence between Ac and UC groups and c) to compare the difference in patient initiated AI discontinuation between Ac and UC groups.

STATS/TARGET ACCRUAL: This is a randomized controlled phase II study with the goal to compare reduction in BPI-SF score for patients treated with Ac vs UC alone. Patients are randomized 1:1 between the two arms. Prior work has indicated a 2-point BPI-SF score reduction as clinically meaningful. A sample size of 56 patients yields a study with 90% power and a one-sided significance level of 0.025 to determine a difference in pain reduction ≥ 2 . These calculations are based on the assumptions of normal distribution of the improvement in BPI-SF score and a standard deviation that is conservatively calculated to be 2.3. An additional 6 patients will be accrued to account for attrition.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-02-05

Title: NCI-2016-00367: A phase IIB study of neoadjuvant ZT regimen (enzalutamide therapy in combination with weekly paclitaxel) for androgen receptor (AR)-positive triple-negative breast cancer (TNBC)

Fujii T, Lim B, Helgason T, Hess KR R, Gilcrease MZ Z, Willey JS S, Tripathy D, Litton JK K, Moulder S, Krishnamurthy S, Yang W, Reuben JM M, Symmans WF F and Ueno NT T. The University of Texas MD Anderson Cancer Center, Houston, TX; The University of Texas MD Anderson Cancer Center; The University of Texas MD Anderson Cancer Center; The University of Texas MD Anderson Cancer Center and The University of Texas MD Anderson Cancer Center.

Body: BACKGROUND: Approximately 50% of TNBC expresses AR by immunohistochemical (IHC) staining. Luminal androgen receptor (LAR) subtype is heavily enriched in hormonally regulated genes, yet negative for ER by IHC. LAR is associated with low pCR rates and long survival. Preclinical data have shown that taxanes inhibit translocation of AR from the cytoplasm to the nucleus where AR is activated. Combining paclitaxel with enzalutamide may inhibit the AR pathway synergistically thereby increasing pCR rates. We hypothesized that patients with AR-positive TNBC who have chemo-insensitive disease (CID) after initial anthracycline-based chemotherapy treated with ZT would have higher RCB-0 and RCB-I rates than those who receive conventional taxane-based chemotherapy. Our team developed a clinical trial to identify patients with CID (**ARTEMIS: A Randomized, TNBC Enrolling trial to confirm Molecular profiling Improves Survival**). In the ARTEMIS trial, treatment-naïve patients with localized TNBC undergo a pretreatment biopsy and then begin anthracycline-based chemotherapy. Molecular testing results and radiographic response assessment are used to identify CID and will guide the second phase of neoadjuvant chemotherapy (NACT) to overcome CID.

PRIMARY OBJECTIVE: To determine RCB-0 and RCB-I rates of patients with TNBC who have CID to initial anthracycline-based chemotherapy and who received ZT.

TRIAL DESIGN AND STATISTICAL METHODS: Patients with CID from the ARTEMIS trial can enroll in the 12-week ZT (paclitaxel, 80 mg/m² intravenously per week; enzalutamide, 160 mg orally per day). We will define pCR (RCB-0) or RCB-I as a response, using a Simon optimal 2-stage design with alpha=beta=10% and then setting the threshold for an acceptable pCR or RCB-I rate at 20%. We will enroll 12 patients into the first stage. If no patients experience pCR or RCB-I, we will stop the study after the first stage. If at least 1 patient experiences pCR or RCB-I, we will enroll 25 more patients for a total of 37 patients. We would declare the treatment worthy of further study if at least 4 of the 37 patients experience pCR or RCB-I. This design has a 54% probability of early termination after the first stage if the true pCR or RCB-I probability is 5%. Because patients with CID have a very low chance (5%) of achieving pCR with additional chemotherapy, improving pCR rates to 20% in this patient population would be clinically meaningful.

BRIEF ELIGIBILITY CRITERIA: *Inclusion criteria:* Primary invasive TNBC patients who have CID under the ARTEMIS trial; AR+ ≥1% nuclear staining by IHC; and adequate physical, organ, bone marrow, and cardiac functions. *Exclusion criteria:* Pregnant or lactating patients, history of colitis or absorption abnormality, known or suspected brain metastasis or leptomeningeal disease, or history of seizure.

CORRELATIVE SCIENCE: Enumeration of circulating tumor cells (CTCs) and expression of CTC-related gene transcripts will be measured to correlate CTC characteristics and/or gene profiles related to the AR pathway and treatment response to ZT.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-02-06

Title: Femara plus ribociclib or placebo as neo-adjuvant endocrine therapy for women with ER+, HER2-negative early breast cancer - The Feline trial

Khan QJ J, Prochaska LH H, Mohammad J, Yuan Y, O'Dea A, Bardia A, Wisinski K, Hard M, Baccaray S, Makhoul I, Wagner J, Laura S, Ma C and Sharma P. University of Kansas Medical Center, Kansas City, KS; University of Miami Health System, Miami, FL; City of Hope, Duarte, CA; Massachusetts General Hospital, Boston, MA; University of Wisconsin, Madison, WI; University of Arkansas for Medical Sciences, Little Rock, AR and Washington University Medical School, St. Louis, MO.

Body: Background:

In early ER+ breast cancer, neo-adjuvant (NA) endocrine therapy (ET) may identify a subset of patients with endocrine sensitive disease with excellent outcomes without chemotherapy. In patients receiving a NA aromatase inhibitor, on-therapy, short term (day 14) Ki-67 of <10% and post NA pre-operative endocrine prognostic index (PEPI) 0 at surgery are associated with low relapse rates without chemotherapy. Ribociclib, a novel CDK4/6 inhibitor is active in ER+ metastatic breast cancer. We hypothesize that ribociclib+letrozole as NA ET for stage II-III breast cancer will increase the number of women with a PEPI 0 at surgery.

Trial Design:

Randomized, placebo-controlled, multi-center, phase II, investigator initiated trial of NA letrozole +/- ribociclib in postmenopausal women with ER+, HER2-, breast cancer. Subjects will be randomized 1:1:1 to letrozole 2.5 mg daily + placebo, letrozole 2.5mg daily + ribociclib 600mg daily on D1-21 of a 28 day cycle (intermittent dosing), or letrozole 2.5mg daily + ribociclib 400mg daily (continuous dosing). Treatment will be continued for 6 months followed by surgery. Research core biopsies and blood will be collected at baseline, at day 14, and at surgery. A Ki67 >10% at day 14 will result in discontinuation of the subject from the protocol as this may be an early indicator of resistance to endocrine therapy. An MRI will be done after 2 months of therapy to assess response/progression. Primary endpoint is a PEPI score of 0 at surgery.

Key Eligibility Criteria:

Postmenopausal (natural or surgical) women with stage II/III ER+, HER2- breast cancer. Must have a palpable breast mass of at least 2 cm. Multicentric/contralateral invasive disease not allowed. Ipsilateral/contralateral DCIS is allowed. Inflammatory breast cancer is excluded.

Specific Aims:

Primary objective: To determine if ribociclib+letrozole as a 24 week NA ET increases rate of PEPI score of 0 at surgery compared to letrozole. Secondary objectives: To determine if ribociclib+letrozole as a 24 week NA ET increases the proportion of tumors with complete cell cycle arrest compared to letrozole; to determine if ribociclib in combination with letrozole for 24 weeks results in improved 5 year RFS compared to letrozole; to examine differences in response rates between the two ribociclib containing arms vs letrozole.

Statistical Methods:

The two ribociclib containing arms (n=80) will be combined for analysis against placebo + letrozole (n=40). Assuming that addition of ribociclib will increase the rate of PEPI 0 by 20%, and setting Type I error rate at 10% and Type II error rates at 20% in the final analysis, a sample size of 80 women in the treatment arms (40 in each arm) and 40 women in the control arm are needed to show significance.

Patient accrual and target accrual:

Participating sites include The Univ of Kansas Med Ctr, City of Hope National Med Ctr, Massachusetts General Hospital, University of Miami Sylvester Comprehensive Cancer Ctr, University of Arkansas for Medical Sciences, and University of Wisconsin. The trial has accrued 16 patients with a target accrual of 120 patients. Accrual should be complete in 2/2017.

Contact information: Qamar Khan, MD (qkhan@kumc.edu).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-02-07

Title: A phase II study to compare fulvestrant (F) 500mg plus placebo versus (vs) F 500mg plus palbociclib (P) as first line treatment for postmenopausal women with hormone receptor (HR)-positive advanced breast cancer (BC) sensitive to endocrine therapy (ET). *“The FLIPPER study”* (GEICAM/2014-12)

Moreno F, Martínez-Jañez N, Garau I, Guerra JA A, Alarcón J, Bermejo B, Gonzalez-Cortijo L, Bueno C, Lao J, Bezares S, Rosell L, Blanch A, Caballero R, Carrasco E, Rojo F, Martín M, O'Connor M, Hernando A and Albanell J. Hospital Clínico Universitario San Carlos, Madrid, Spain; Hospital Universitario Ramón y Cajal, Madrid, Spain; Hospital Son Llátzer, Palma de Mallorca, Spain; Hospital Universitario de Fuenlabrada, Madrid, Spain; Hospital Son Espasses, Palma de Mallorca, Spain; Hospital Clínico Universitario de Valencia, Valencia, Spain; Hospital Universitario Quirón Madrid, Madrid, Spain; Hospital Universitario Infanta Cristina, Madrid, Spain; Hospital Universitario Miguel Servet, Zaragoza, Spain; GEICAM (Spanish Breast Cancer Group), Madrid, Spain; Fundación Jiménez Díaz, Madrid, Spain; Instituto de Investigación Sanitaria Gregorio Marañón-Universidad Complutense de Madrid, Madrid, Spain; ICORG (Cancer Trials Ireland), Ireland and Hospital del Mar - IMAS, Barcelona, Spain.

Body: Background: Delaying/overcoming resistance to ET in HR-positive HER2-negative BC patients (pts) is a major need to further improve safe and efficacious treatment options. F is a selective estrogen receptor (ER) downregulator currently indicated for the treatment of HR-positive metastatic BC in postmenopausal women with disease progression following anti-estrogen therapy. In FIRST trial F 500mg improved median time to progression (TTP) over anastrozole (23.4 vs 13.1 months, respectively) in untreated metastatic BC. P is a selective reversible inhibitor of cyclin-dependent kinase 4/6. FDA granted its accelerated approval based on progression-free survival (PFS) in combination with letrozole for postmenopausal women with ER-positive and HER2-negative advanced BC as initial ET (PALOMA-1). In another study, after progression to ET, P in combination with F resulted in longer PFS than F alone (PALOMA-3).

The high median TTP achieved with F alone (FIRST) coupled with the significant benefit of adding P to F (PALOMA-3) suggest that F 500mg in combination with P in the first-line setting may significantly improve long-term disease control.

Trial Design: This is an international, randomized, double-blind, multicentre phase II study comparing F 500mg in combination with P vs F 500mg plus placebo as first line therapy in postmenopausal women with HR-positive, HER2-negative metastatic BC who have received ≥ 5 years of adjuvant ET for early disease and remained disease free for >12 months following its completion or have “de novo” metastatic disease. HR and HER2 status will be based on central testing on the most recent tumour biopsy. Patients will be randomized 1:1. The primary objective is to compare the efficacy of both treatment arms in terms of PFS at 1 year according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 by investigator assessment. As secondary efficacy objectives, PFS, Objective Response Rate (ORR), Clinical Benefit Rate (CBR), Overall Survival (OS), and 1-year and 2-year survival probabilities, have been considered. Other secondary objectives include the comparison of safety, tolerability and health-related quality of life between the treatment arms. As exploratory objectives, the identification of promising biomarkers related with response to study therapy and primary/acquired drug resistance. Pts will be stratified by the site of disease (visceral vs non-visceral) and disease presentation at study entry (recurrent disease vs metastatic “de novo”). With a sample size of 190 pts, the analysis would have 80% power to detect a difference between both treatment arms, assuming PFS proportions of 0.545 and 0.695, respectively. This study is sponsored by GEICAM and Cancer Trials Ireland (formerly ICORG) is also participating. Recruitment started in February 2016 with 14 pts included. Analysis of the primary endpoint is planned for Q1 2018. ClinicalTrials.gov identifier: NCT02690480.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-02-08

Title: Open label, phase 2 safety, efficacy, and pharmacokinetic study of pre-surgical intramuscular and intraductal fulvestrant in women with invasive breast cancer or DCIS undergoing mastectomy or lumpectomy

Feldman S, Gomberawalla A, Alonso A, Rea J, Quay S and Lawrence R. Columbia University Medical Center, New York, NY and Atossa Genetics, Seattle, WA.

Body: BACKGROUND: Breast cancer (BC) is the leading cause of cancer in women in the United States and the second leading cause of cancer-related death. Currently available options for prevention are oophorectomy, bilateral mastectomy, or medical therapy, such as tamoxifen, raloxifene or aromatase inhibitors. None of these options for prevention are without significant side effects with low patient uptake. This study proposes fulvestrant instilled directly into the breast via the nipple orifices as intraductal therapy. Providing a local therapy into the ducts could reduce the morbidity associated with prevention while potentially better targeting the carcinoma cell. Building on prior studies with cytotoxic agents, this study utilizes the pure anti-estrogen fulvestrant to be injected in up to 5 ducts to determine its effect on BC as well as the safety of this method of administration.

TRIAL DESIGN: An open-label, non-randomized PK study of pre-surgical fulvestrant in women scheduled for mastectomy or lumpectomy. Eligible subjects will be undergoing mastectomy or lumpectomy. The first 6 subjects will be treated with the standard dose of 500 mg intramuscular fulvestrant to establish the reference PK curve. The subsequent 24 subjects will receive fulvestrant by intraductal instillation. Subjects where at least 1 suitable duct is identified will undergo nipple aspiration in order to facilitate duct identification and intraductal infusion of a fulvestrant. A maximum of 5 ducts will receive intraductal infusion of fulvestrant. Across all ducts, the total dose will not exceed 500 mg (10 mL). Subsequent to mastectomy or lumpectomy subjects will undergo serial blood draws to determine fulvestrant blood concentration levels and tissue drug levels.

ELIGIBILITY: Female, age \geq 18, mastectomy/lumpectomy scheduled within 30 days, Stage 1/2, ER+ low grade invasive BC or DCIS, ECOG scale 0-1.

Primary Objective: The safety and tolerability of intraductal administration of fulvestrant in women with Stage 1 or 2 invasive ductal carcinoma or DCIS, prior to mastectomy or lumpectomy.

Secondary Objectives: The pathological effects, specifically changes in Ki67, ER/PgR expression between pre-fulvestrant biopsy and post-fulvestrant surgical specimen.

STATISTICAL METHODS: Aim of this study is to assess safety and tolerability in subjects receiving intraductal fulvestrant. In addition this trial aims to characterize the PK of this alternative route of administration, and compare the intraductal PK profile to that of standard intramuscular administration. Finally, the 6 study subjects who receive intramuscular fulvestrant will be qualitatively compared with the main study cohort of 24 subjects who receive intraductal fulvestrant. Change in Ki67 labeling index will be compared between the two time points (baseline or time of diagnostic biopsy v. time of the surgically excised specimen collection).

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2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-02-09

Title: Phase II pre-surgical window trial of telapristone acetate (TPA) in early breast cancer and DCIS patients: Distribution of TPA in plasma, normal breast tissue and tumors

Lee O, Muzzio M, Ivancic D, Rogers C, Allu S and Khan SA A. Northwestern University, Chicago, IL and Illinois Institute of Technology Research Institute, Chicago, IL.

Body: Background: In vitro and preclinical data support the notion that anti-progesterone therapy will have activity against both estrogen and progesterone receptors (ER/PR) positive and negative breast cancer, but biomarkers of efficacy may differ in different types of breast cancer. We have conducted a pre-surgical window trial of oral telapristone acetate (TPA, CDB-4124) treatment in early breast cancer patients. This first ever trial of oral TPA for breast cancer and DCIS patients will provide us pilot biologic data that will help select the right population and the right biomarkers for future trials. Here we report distribution of TPA in plasma and breast normal tissue and tumors collected at surgery.

Methods: our trial was a 1:1 randomized, double-blind, and placebo-controlled pre-surgical window trial of oral TPA 12mg (Proellex, CDB-4124, Repros Therapeutics Inc.) treatment for 2-10 weeks. 70 pre and postmenopausal women undergoing surgery for Stage 0-II breast cancer were recruited to the study. The surgical samples of 61 patients were used to determine the concentrations of TPA and its mono-demethylated metabolite (dTPA, CDB-4453) in plasma and matched normal tissue and tumor by Liquid chromatography–tandem mass spectrometry at the Illinois Institute of Technology, while maintaining the blind for the primary endpoint of cell proliferation. Statistical significance and analysis were calculated by Wilcoxon matched-pairs signed rank test and non-parametric Spearman correlation.

Results: We found that 32/61 women displayed detectable plasma concentrations of TPA and dTPA (median with IQR) 109ng/mL (71.3, 216) and 46.5 ng/mL (34.2, 73.7), respectively. TPA concentration was 2.3 times higher than dTPA in plasma ($p < 0.0001$). The normal and tumor tissue samples of these 32 women were further analyzed. In normal tissue samples, the concentrations of TPA and dTPA were 283 ng/g (70.7, 326) and 51.0 ng/g (24.4, 122), respectively. TPA concentration was 5.5 fold higher than dTPA in normal tissue ($p < 0.0001$). In tumors, the TPA and dTPA concentrations were 137 ng/g (31.1, 278) and 36.4 ng/g (17.3, 68.7), respectively. TPA concentration was 3.8 fold higher than dTPA in tumors ($p < 0.0001$). Interestingly, TPA and dTPA were more abundant in normal tissue than in tumors ($p = 0.0005$ for TPA, and $p = 0.0013$ for dTPA). We found that TPA and dTPA was highly correlated in plasma ($r = 0.492$, $p = 0.0042$). Plasma TPA concentration was highly correlated with normal tissue concentration ($r = 0.61$, $p = 0.0003$) but non-significantly correlated with tumor concentration ($r = 0.32$, $p = 0.147$). However, the normal and tumor tissue concentrations of TPA and dTPA were highly correlated ($r = 0.71$, $p = 0.0002$ for TPA and $r = 0.556$, $p = 0.0072$ for dTPA).

Conclusions: Plasma TPA concentrations reflect concentration in normal breast tissue better than in tumors. However, within the breast, TPA concentration in normal and tumor tissue is correlated. Our trial is to be unblinded shortly, and we plan to relate these results to the proliferative rates in tumor and normal tissue. The variability observed in plasma and tissue concentrations also suggests that pharmacogenomics studies may be appropriate in the future.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-03-01

Title: MADELINE: A prospective observational study of mobile app-based patient reported outcomes in advanced breast cancer

McRoy LL L, Mitra D, Hollis K, Kaye JA A, Zelnak A and Cheyl J. Pfizer, New York, NY; RTI Health Solutions, Durham, NC; Atlanta Cancer Care, Cumming, GA and Georgia Cancer Specialists, Macon, GA.

Body: BACKGROUND: There have been few studies evaluating the day-to-day effects of advanced breast cancer (ABC) and its treatment on patients in a real-world setting. Palbociclib is a novel CDK4/6 inhibitor approved in the US for hormone-receptor positive, human epidermal growth factor receptor negative (HR+, HER2-) ABC/metastatic breast cancer (MBC) in combination with letrozole as initial endocrine based therapy in postmenopausal women or with fulvestrant in women with disease progression following endocrine therapy. With the introduction of this first-in-class drug it is important to understand the experiences of patients initiating ABC therapies including palbociclib in real-world settings and to document the management of these therapies. A smartphone-based mobile application has been developed to collect patient-reported outcome (PRO) data to assess the impact of ABC and associated treatment on symptoms, functioning and quality of life (QOL) as reported by patients. The application is further designed to provide patients initiating palbociclib with a virtual community to connect to others enrolled in the study for peer support. Additionally, clinical data on therapy management (e.g. dose modifications, interruptions, discontinuations, adverse event management and monitoring) will be obtained from patients' medical records to explore the association between patient reported functioning and neutropenia.

Study Design: A prospective, observational, non-interventional study. PROs collected via a mobile application and clinical data via case report forms completed by investigator.

Eligibility Criteria: Women with HR+/HER2- ABC receiving palbociclib in combination with letrozole or fulvestrant as per US label (Group 1) or approved therapies for ABC other than palbociclib (Group 2). No comparison is intended between the 2 groups.

Specific Aims: The primary goals are to describe changes in patients' general health status as measured by monthly administration of the 12-Item Short Form Health Survey, describe changes in patients' psychological distress as measured by monthly administration of the Center for Epidemiological Studies Depression Scale, and describe the extent to which ABC and its treatment are associated with changes in patients' lives in terms of symptoms, functioning and QOL as measured by daily and weekly administration of targeted patient-reported questions. Additionally, for patients who are employed at baseline, time lost from work in relation to breast cancer and its treatment will be quantified. Data from patients' medical records will be used to document changes in therapy as well as the incidence, severity, and duration of neutropenia. The association between patient reported functioning and neutropenia will also be assessed. Finally, real-world monitoring patterns will be assessed.

Statistical Methods: Descriptive statistics will be used to summarize all endpoints. Meta-data regarding use of virtual community resources will be explored for relationships to PRO data.

Present Accrual and Target Accrual: Approximately 450 patients from up to 30 US sites will be enrolled. Study duration will be approximately 12 months assuming 6 months of recruitment. It is expected the study will start enrollment Q3 2016.

Sponsor: Pfizer.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-03-02

Title: Maximising recruitment and retention of patients into UK-ANZ POSNOC trial

Goyal A, Coleman RE E, Dodwell D, Fallowfield L, Jenkins VA A, Mann B, Reed MW W and POSNOC Trial Management Group. Royal Derby Hospital, Derby, United Kingdom; Weston Park Hospital, Sheffield, United Kingdom; St James Hospital, Leeds, United Kingdom; SHORE-C, Falmer, United Kingdom; Royal Melbourne and Royal Women's Hospital, Victoria, Australia and Brighton and Sussex Medical School, Brighton, United Kingdom.

Body: The success of a clinical trial is often dependant on whether recruitment targets can be met in the required time frame. Surgeons want to gather robust evidence but may experience considerable discomfort in relation to their clinical instincts and concerns about patient eligibility and safety. The ongoing POSNOC study design is pragmatic to maximise recruitment. A patient information DVD is used as an adjunct to patient information leaflet. The protocol allows for either axillary radiotherapy or axillary node clearance. Here we report recruitment and retention of participants into POSNOC, the associated barriers and challenges, and various strategies employed to overcome these barriers.

A total of 368 participants were recruited till May 2016. A total of 90%(331) of participants were recruited via non intra-operative pathway, and 10%(37) from intra-operative pathway. The most common known reasons for non-participation of eligible women were - woman wanting axillary treatment and clinician/MDT deciding woman needs axillary treatment. The mean randomisation yield from screening was 30% (range 6 to 100%). The highest recruiting sites were Derby, Manchester, Oxford, Belfast and Bristol.

The screening logs identified barriers as: fewer than estimated eligible women, clinicians not offering trial to all eligible patients and patient acceptability.

To reach recruitment targets in a timely fashion, a multifaceted approach is being employed. a) Protocol amendments to widen the inclusion criteria, b) 250 patients to be recruited by Australia and New Zealand sites, c) additional 50 sites to be opened in the UK, d) encourage OSNA centres to follow the non intra-operative pathway, e) communication workshops, f) POSNOC poster in breast units to raise awareness, g) questionnaire survey - sites to identify local issues and their perceptions about recruitment, h) social media - POSNOC WhatsApp group i) newsletters and briefings, j) competitions, k) tips to maximise recruitment document that includes a template script of one way to introduce the study l) regional telephone conferences for research nurses, m) investigator meeting.

The success of these strategies remains to be assessed and shall be reported separately.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-03-03

Title: Challenges faced across borders to open European academic multicentre projects: The ET-FES program part of the ERA-Net TRANSCAN JTC 2011

Brain E, Corradengo D, Oriana N, Piccardo A, Matteucci F, Cortes J, Harbeck N, Würtle R, Piris A, Alberini J-L, Merlo DF, Degenhardt T, Turbiez I, Madar O, Monti M, Cesaro A, Rivitti E, Rollandi GA, Iacozzi M, Campazzi E, Campora S, Camporese D and Gennari A. Institut Curie / Hôpital René Huguenin, Saint-Cloud, France; E.O. Ospedali Galliera, Genova, Italy; IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori - I.R.S.T., Meldola, Italy; Vall d'Hebrón Institute of Oncology, Barcelona, Spain; University of Munich, Frauenkliniken Maistrasse-Innenstadt und Großhadern, München, Germany; IRCCS Azienda Osp. Univ. San Martino- IST, Genova, Italy; IBSA Institut Biochimique SA, Lugano, Switzerland; Covance, Milano, Italy and Advanced Accelerator Applications AAA, St Genis Pouilly, France.

Body: Background

The activation of international non-profit clinical trials funded by the European Commission (EC) is challenging given the cross-borders regulations and the need to follow specific timelines according to EC rules. We report here the logistic procedures and challenges faced by 4 academic centres from 4 different countries from the European Union (EU) for the activation of such program in metastatic breast cancer (MBC).

Materials and Methods

The primary objective of the ET-FES program is to validate the use of a new radiotracer ¹⁸F Fluoroestradiol (FES), targeting estrogen receptors, as a tool to better predict endocrine responsiveness in MBC, with PET/CT. The trial is sponsored by EO Galliera (Genoa, Italy) and brings together Italy, Spain, France and Germany. In 10/2012, ET-FES was approved for funding from EC under the Seventh Framework Programme after the first ERA-NET TRANSCAN Joint Transnational Call (JTC) for Proposals (2011) on validation of new biomarkers for personalized cancer medicine.

Results

The official start of the program was set up on 06/2013 by the Italian Ministry of Health. Time to institutional review board and to competent authority (CA) approvals were 1.5 and 11, 2 and 5, 3 and 16, and 13 and 14+ months in Italy, France, Spain and Germany respectively. Overall, no blocking comment was raised by the ethical committees; only minor clinical and methodological issues were raised in Germany and Spain. Issues from CA were raised in all countries except France (12, 21 and 23 queries in Italy, Spain and Germany respectively), on quality aspects of ¹⁸F-FES investigational medicinal product dossier. At the sponsor level, time to final agreement signature with Advanced Accelerator Applications, the ¹⁸F-FES manufacturing company, required 13 months. First patient could be enrolled in Italy 14 and 22 months after ethical committee approval and after the official start of the ET-FES project respectively.

Conclusions

As of May 2016, of 310 patients expected, only 28 have been enrolled from Italy. From a regulatory viewpoint and acknowledging that ¹⁸F-FES does not have yet any marketing approval in the EU, the ET-FES program approval process was timely completed at the ethical committee level in Italy, France and Spain. Time to CA approval varied across countries and was timely achieved only in France, due to requirements varying from CA to CA, stressing the serious lack of harmonized procedures although intended by the 2001/20/EC directive. Regarding sponsor's responsibilities (i.e. Italy), the administrative procedures needed to activate this type of EU projects remain critical, appealing for more tolerant time span in order to satisfy all the legal aspects on contracts by public bodies, according to national rules and laws. One needs to be very conscious of these timelines when applying to EU/EC calls, especially when the time allowed for the conduction of research is limited (3 years here), unless jeopardizing the entire multicentre and multinational effort.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-04-01

Title: BRE12-158: A phase II randomized controlled trial of genomically directed therapy after preoperative chemotherapy in patients with triple negative breast cancer (TNBC)

Schneider B, Miller KD D, Badve S, O'Neil B, Helft P, Chitambar C, Falkson C, Nanda R, McCormick M, Danso M, Blaya M, Langdon R, Lippman M, Paplomata E, Walling R, Thompson M, Robin E, Aggarwal L, Shalaby I, Canfield V, Adesunloye B, Lee T, Daily K, Ma C, Erban J, Radhakrishnan N, Bruetman D, Graham M, Reddy NA A, Lynce FC C and Radovich M. Indiana University Simon Cancer Center; Medical College of Wisconsin; University of Alabama Birmingham; University of Chicago; Meritus Center for Clinical Research; Virginia Oncology Associates; Memorial Cancer Center; Nebraska Methodist Hospital; University of Miami; Winship Cancer Institute of Emory University; Community Regional Cancer Care; Aurora Health Care; Community Healthcare System; Fort Wayne Medical Oncology and Hematology; Joe Arrington Cancer Research and Treatment Center; Mercy Clinic Oklahoma Communities; IU Health Arnett; IU Health Goshen Center for Cancer Care; Pinnacle Health Cancer Center; University of Florida; Washington University at St. Louis; Tufts Medical Center; University of Cincinnati; Erlanger Health System; Community Hospitals of Anderson and Madison Co and Georgetown University.

Body: Background: About 1/3 of patients with TNBC who receive preoperative therapy will experience a pathological complete response (pCR). Patients with residual disease have a markedly inferior overall survival (OS) compared to those who experience pCR. Recently, the CREATE-X trial demonstrated an improvement in disease free survival (DFS) and OS for post-neoadjuvant capecitabine; although the addition of capecitabine to standard therapy has not previously improved outcome across other non-selected adjuvant or neo-adjuvant trials. Prior data have also demonstrated that the residual tumors are genomically diverse and that these genetic changes are reflected at time of relapse.

Trial Design: This trial is a randomized phase II trial to determine whether a genomically guided therapy in the setting of incomplete response to standard neoadjuvant therapy will improve outcomes compared to standard of care. DNA from archived tumor samples collected at the time of surgery will be extracted and sequenced. The sequencing data will be interrogated for known genomic drivers of sensitivity or resistance to existing FDA approved agents. A cancer genomic tumor board (CGTB) will consider the genomic data along with the patient's prior treatment history, toxicities, and comorbidities and select the optimal therapy. Participants with a CGTB recommendation will be randomized to Experimental Arm A (genomically directed monotherapy) or Control Arm B (standard of care). Participants may have no CGTB recommendation either because sequencing did not identify a matched drug or because the drug was contraindicated and will be assigned to Control Arm B.

Eligibility criteria: Patients must have histologically confirmed TNBC with completion of all definitive local therapy and no evidence of metastatic disease. There must be significant residual disease characterized by >2cm primary tumor, or lymph node positivity or RCB classification II or III. An FFPE tumor block with tumor cellularity >20% is required. All patients must have completed preoperative chemotherapy including a taxane or anthracycline or both.

Specific aims: The Primary Aim is to compare 2-year DFS with a genomically directed therapy vs. standard of care. Secondary Aims include 1-year DFS, 5-year OS, collection of archival specimens for correlative studies, and to describe toxicities. Exploratory Aims are to describe the evolution of genomically directed therapies during the course of the study and to evaluate the drug specific effect on efficacy and toxicity.

Statistical methods: In order to detect an improvement in the fraction of patients free from disease at 2-year from 40% in the control Arm B to 63.2% in the genomically directed Experimental Arm A (corresponding to an HR=0.5), 136 participants will have 80% power to detect a difference in DFS using a two-side log-rank test with 0.05 level of significance.

Present accrual/target accrual: 38 accrued of 136 to be randomized.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-04-02

Title: DETECT III and IV – Individualized CTC-based therapy of metastatic breast cancer

Polasik A, Schramm A, Friedl TWP WP, Rack B, Trapp E, Fasching PA A, Taran F-A, Hartkopf A, Schneeweiss A, Müller V, Aktas B, Pantel K, Meier-Stiegen F, Wimberger P, Janni W and Fehm T. Gynecology and Obstetrics, University Hospital Ulm, Ulm, Germany; Gynecology and Obstetrics, Klinikum der Ludwig-Maximilians-Universität, Munich, Germany; Gynecology and Obstetrics, University Hospital Erlangen, Erlangen, Germany; Gynecology and Obstetrics, University Hospital Tübingen, Tübingen, Germany; University Hospital Heidelberg, Ulm, Germany; Gynecology and Obstetrics, University Hospital Hamburg-Eppendorf, Hamburg, Germany; Gynecology and Obstetrics, University Hospital Essen, Essen, Germany; University Hospital Hamburg-Eppendorf, Hamburg, Germany; Gynecology and Obstetrics, Heinrich-Heine-Universität, Düsseldorf, Germany and Gynecology and Obstetrics, University Hospital Dresden, Dresden, Germany.

Body: Background: Circulating tumor cells (CTCs) are found in patients with early and metastatic breast cancer (MBC), and both their prognostic and predictive value has been described already. There is growing evidence that CTC phenotype may differ from the primary tumor. However, CTC targeted therapy is not used in clinical routine, and treatment decisions often still are based on the primary tumor's phenotype without considering CTC-characteristics. The aim of the DETECT studies is to investigate and evaluate the role of presence and phenotype of CTC for guiding therapeutic decisions in women with HER2-negative MBC.

Trial design and eligibility criteria: In a joint screening for DETECT III and IV, women with HER2-negative MBC are tested for CTCs and their HER2-phenotype. CTC detection is performed by the FDA-approved CellSearch System® (Janssen Diagnostics, Raritan, USA).

Patients with HER2-positive CTCs are randomized in the multicenter Phase III study DETECT III to a physician's choice chemo- or endocrine therapy with or without additional HER2-targeted treatment with lapatinib.

Women with only HER2-negative CTCs are recruited to the multicenter open-label phase II study DETECT IV. Postmenopausal women with hormone-receptor positive MBC are treated with everolimus and a physician's choice endocrine therapy in DETECT IVa. Patients with hormone-receptor positive MBC and an indication for chemotherapy and patients with triple-negative MBC receive mono-chemotherapy with eribulin in DETECT IVb.

Treatment efficacy will be evaluated based on the early available CTC clearance rate (in DETECT III and DETECT IVa) and progression-free survival (in DETECT IVb) respectively, as the primary endpoint; secondary objectives will be to estimate disease control rate, progression-free (DETECT III and IVa) and overall survival, toxicity and tolerability of treatments with lapatinib, everolimus and eribulin, and quality of life.

Specific aims: Changes in CTC-dynamics during therapy and their HER2-phenotype may influence following therapy decisions. The DETECT studies evaluate the prognostic and predictive role of CTCs as well as the efficacy of CTC based therapy to enable the establishment of a more personalized therapy for patients with MBC that might lead to prolonged progressive free survival and/or improved quality of life. The accompanying translational research programs investigate various markers for molecular characterization of CTCs and prediction of therapy response.

Present accrual and target accrual: More than 1550 patients with HER2-negative MBC have already been screened within the DETECT study program. Thus, it is the worldwide largest study concept with therapy decisions resulting from CTC-testing and CTC-phenotypization.

Contact: For further information on the DETECT study program please contact www.detect-studien.de or studienzentrale.ufk@uniklinik-ulm.de.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-04-03

Title: The impact of the 21 gene recurrence score (RS) on chemotherapy prescribing in estrogen receptor (ER) positive, lymph node positive early stage breast cancer in Ireland

Keegan NM M, Milewski M, Kelly CM M, Murphy V, Chao C, Walsh J, Kennedy MJ John, O'Connor M, Murphy C, O'Reilly S, Keane M, Duffy K, Hennessy B and Morris PG G. Beaumont Hospital, Dublin 9, Ireland; Mater Misericordiae University Hospital, Dublin 7, Ireland; Cancer Trials Ireland, Dublin 2, Ireland; Genomic Health, Ireland; St James's Hospital, Dublin 8, Ireland; University Hospital Waterford, Waterford, Ireland; Bon Secours, Cork, Ireland; Cork University Hospital, Ireland; University College Hospital Galway, Ireland; Letterkenny General Hospital, Ireland and AMNCH and St Vincent's University Hospital, Dublin, Ireland.

Body: BACKGROUND

For Estrogen Receptor (ER) positive, early stage breast cancer, the 21 gene Recurrence Score (RS) has clinical use both as a prognostic tool and to predict chemotherapy benefit. The availability of this tool in Ireland has led to a reduction in the use of adjuvant chemotherapy for women with lymph node (LN) negative disease. However, the RS is not routinely funded for patients with LN positive (LN+) breast cancer in Ireland. In addition, there are limited international data on the use of this tool in the preoperative setting. In this prospective observational study, we are investigating whether access to the 21 gene RS leads to a reduction in the receipt of chemotherapy for patients with ER+, LN+ breast cancer, and to correlate the 21 gene RS with response to preoperative systemic therapy.

TRIAL DESIGN

This is a national, multi-site, prospective, observational study that will examine the impact of the 21 gene RS on chemotherapy recommendations in both the neoadjuvant and adjuvant setting. Prior to and following tumor testing with the 21 gene RS, Physicians will complete a questionnaire which details type and strength of systemic therapy recommendations.

ELIGIBILITY

Cohort 1 (postoperative) will include patients with ER+ tumors of any size with involvement of 1-3 lymph nodes (N1 including micrometastases). Cohort 2 (preoperative) will include patients with ER+, T2-T4 tumors with biopsy proven nodal metastases. Both cohorts will have ECOG PS 0 or 1 and be fit for consideration of chemotherapy as determined by the Investigator.

SPECIFIC AIMS

The primary endpoint is the percentage reduction in the number of patients for whom treating physicians recommend chemotherapy after testing with 21 gene RS. Secondary endpoints include the correlation between the 21 gene RS and residual cancer burden score, as well as pathological, clinical and radiological response rates. The economic impact of the 21 gene RS in ER+, LN+ will also be assessed.

STATISTICAL METHODS

The sample size is based on similar decision impact studies conducted in other countries. Physician recommendations for chemotherapy pre 21-gene RS and recommendations post 21-gene RS testing will be compared and percentage change estimated with 95% confidence intervals. For secondary endpoints, the Pearson correlation coefficient (ρ) will be used to examine the strength of the correlation between the 21 gene RS category and response. A budget impact model will be used to estimate the cost reduction in adjuvant chemotherapy as a result of 21-gene RS testing.

PRESENT ACCRUAL AND TARGET ACCRUAL

Target accrual is 75 in each of the neoadjuvant and adjuvant cohorts to total 150 patients.

Supported by Genomic Health.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-04-04

Title: 21-gene recurrence score® in patients with primary metastatic ER+ HER2- breast cancer

Barinoff J, Anastasiadou L, Brandi C, Junker-Stein A, Silbermann J, Langenfeld M, Fortmann C and Thill M. Agaplesion Markus Krankenhaus, Frankfurt am Main/Germany; Klinikum Frankfurt Höchst, Frankfurt am Main/Germany and Genomic Health, Genève, Switzerland.

Body: Background and Purpose

Results from the 21-gene Recurrence Score (RS) are prognostic and predictive for recurrence in early breast cancer independent of clinical-pathological parameters. Prognostic information and distribution of RS in primary metastatic breast cancer (PMBC) was reported in a previous study by King et al but remains ultimately unclear in ER+ HER2- patients. We hypothesize that there is a prognostic correlation between RS and TTP/OS in PMBC independent of clinical-pathological parameters, and the objective of the study is to evaluate the test for that cohort.

Patients and Trial Design

Between 2004 and 2014, 150 patients with first diagnosis of ER+/HER2- PMBC were enrolled in a multicenter, prospectively collected database. Pretreatment breast tumor samples will be analyzed by using 21-gene RS. Minimum follow-up will be 24 months.

Methods and Statistical Analysis

Patient characteristics, time to first progression (TTP), overall survival (OS) and treatment modalities will be described. The association between RS risk groups and both TTP and OS will be assessed by Kaplan-Meier estimates and compared with the log-rank test. Multivariate Cox regression models will be utilized to adjust for covariates. Additionally, assessing correlation of RS results using RS cut offs of 18 and 30 and additionally of 11 and 25 as well as analysis of quantitative ER from Oncotype DX assay for patients treated first-line with endocrine therapy are planned.

First results are expected to be presented in 2017.

Contact information for people with a specific interest in the trial:

Dr. Jana Barinoff

barinoff.jana@googlemail.com.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-04-05

Title: Measuring the impact of MammaPrint on treatment in breast cancer patients: A prospective registry (IMPACT)

Soliman H, Untch S and Blumencranz L. Moffitt Cancer Center, Tampa, FL and Agendia Inc., Irvine, CA.

Body: *Hatem Soliman*¹, *Sarah Untch*², *Lisa Blumencranz*²

1. Moffitt Cancer Center, Tampa, FL

2. Agendia Inc, Irvine, CA

Measuring the Impact of MammaPrint on treatment in Breast Cancer Patients: A Prospective Registry (IMPACT)

Background:

Gene expression profiling in breast cancer offers the potential to improve prognostic accuracy, treatment choice, and health outcomes in women diagnosed with early-stage breast cancer. Numerous gene-profiling assays are now available, which can be applied to a single tumor specimen to provide physicians with a more complete basis for treatment decisions.

- MammaPrint is 70-gene profile to estimate whether patients are at high or low risk of developing metastases within the first 10 years after curative surgery.

- BluePrint is an 80-gene molecular subtyping profile that discriminates between three breast cancer subtypes: Luminal, HER2, and Basal

Trial design:

IMPACT is a prospective, case-only, study to measure the impact of MammaPrint on treatment decisions in breast cancer patients. The primary objective will be to measure the impact of MammaPrint on treatment decisions in stage I and II Hormone Receptor (HR)-positive, HER2-negative breast cancer patients. As a secondary objective, the impact of MammaPrint on treatment will be assessed in patients with T1a/bN0/1 (up to 1 LN) Triple Negative or HER2-positive breast cancer. Baseline clinical data and physician chemotherapy intention before knowing the MammaPrint result will be entered in CRF 1. After completion of CRF1, the MammaPrint result will be released. CRF2 will be completed after the final treatment decision has been made. This CRF will capture physician chemotherapy intention after the MammaPrint result and the impact of these results.

Eligibility:

The study will include women aged ≥ 18 years with histologically proven invasive stage I-II, HR-positive, and HER2-negative breast cancer, OR, T1a/bN0/1 (up to 1 node) triple negative or Her2-positive breast cancer who signed informed consent.

Objectives:

Primary objective:

- Assess the impact of MammaPrint and BluePrint on chemotherapy + endocrine versus endocrine alone treatment decisions in HR-positive, HER2-negative breast cancer patients.

Secondary objectives:

- Assess the impact of MammaPrint and BluePrint on treatment decisions in T1a/b, N0/1 (up to 1 node) triple negative or Her2-positive breast cancer patients

- Compare clinical subtype based on IHC/FISH ER, PR, HER2 and Ki-67 (if available) with BluePrint molecular subtype

Statistical methods:

A sample size of 331 patients is required to detect a 25% overall treatment change (5% significance and 95% power) in stage I and II HR-positive, HER2-negative patients. In addition at least 50 T1a/bN0/1 (up to 1 LN) Triple Negative, and at least 50 T1a/bN0/1 (up to 1 LN) HER2-positive breast cancer patients will be enrolled. A McNemars test will be performed for the comparison of the two proportions of treatment intentions (before and after), both expressed as a percentage.

Accrual: The study started accruing in November 2015. Approximately 15-20 institutions in the United States will participate.

Contact information: Hatem.Soliman@moffitt.org.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-05-01

Title: Three-monthly dynamic evaluation of CEA and CA15-3 (followed by 18-FDG PET) vs usual practice in the follow-up of early breast cancer (BC) patients (pts): A prospective randomized trial (KRONOS-patient-oriented new surveillance study, Italy)

Zamagni C, Gion M, Mariani L, Stieber P, Quercia S, Rubino D, Bernardi A, Cacciari N, Fini A, Lenzi M, Minichillo S, Pizzirani C, Pagliaro M, Tomasini S and Barbieri E. SSD Oncologia Medica "Addarii", Policlinico S. Orsola-Malpighi, Bologna, Italy; Centre for the Study of Biological Malignancy Markers, Venezia, Italy; Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy and Institute of Clinical Chemistry, University of Munich, Munich, Germany.

Body: Background: Current guidelines for BC surveillance in asymptomatic pts recommend annual mammography and periodical physical examination. These recommendations arise from two trials conducted in the 1980's: since then no other randomized controlled trials (RCTs) have been conducted on BC follow-up. However our knowledge on BC biology, diagnosis of metastases and treatment has improved. The aim of this prospective RCT is to verify if the serial measurement of CEA and CA15.3 (followed by 18-FDG PET) can anticipate the diagnosis of breast cancer recurrence compared to control arm. If this intermediate end-point will be met a subsequent extension trial will investigate the impact of the earlier diagnosis of distant metastases on survival. **Methods:** Pts diagnosed with stage I-III BC, who underwent adequate surgery are eligible. Special histologies and low-risk cases according to St. Gallen criteria are excluded. We will include pts at the beginning of the follow-up after the conclusion of primary treatment (cohort 1), and pts that have concluded without relapse the first 5 years of follow-up (cohort 2). Eligible pts will be randomized in a 1:1 ratio to follow-up according to local practice (control arm) or to three-monthly serial dosing of CEA and CA15.3 and subsequent imaging studies (18-FDG PET) only in case of an increase of CEA and/or CA 15.3 greater than a critical difference (CEA +100% and/or CA15.3 +75%) compared to baseline (experimental arm). The following stratification factors will be used: node negative vs positive, HER2 negative vs positive, ER positive vs negative. Eight-hundred pts will be enrolled over 3 years. For such a calculation, we made the assumption of a 20% 5-year incidence of relapse. The target reduction of 3 months in restricted mean survival time (RMST) between the two arms implies a median time of diagnostic anticipation, conditional on having breast cancer recurrence, of 10 months. The follow-up will continue until 10 years from surgery. The first patient was enrolled on 23rd October 2014, up to now 434 pts have been enrolled. The present trial was approved by the Ethical Committee of S. Orsola-Malpighi Hospital and is registered on clinicaltrials.gov (NCT02261389).

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-06-01

Title: KARISMA, The karma intervention study. - A tamoxifen dose determination trial

Borgquist S, Eriksson M, Czene K and Hall P. Lund University, Lund, Sweden and Karolinska Institutet, Stockholm, Sweden.

Body: Background: Breast cancer incidence is increasing throughout the world. Despite remarkable risk reducing effects demonstrated in preventive randomised endocrine trials; primary preventive strategies are scarcely part of clinical routine. Putative reasons for reluctance may be inadequate tools for identification of women at high risk of breast cancer and reluctance to prescribe pharmaceuticals with considerable side effects in the preventive setting.

Trial Design: A randomized, double blinded, six-armed placebo controlled dose determination study to investigate the optimal dose of tamoxifen with the most favorable side effect spectra. To reduce the number of participants, costs and to shorten the trial period; change in mammographic density will be used as clinical outcome. After randomization and baseline mammography, women will be treated with placebo, 1mg, 2.5mg, 5mg, 10mg or 20mg of tamoxifen for six months where after a subsequent mammogram is performed.

Eligibility criteria: 1) Attending the national Swedish mammography screening program, 2) Having a measurable mammographic density, i.e. ≥ 4.5 % density (volumetric) measured by Volpara.

Specific aims: The primary aim is to identify the minimum dose of tamoxifen non-inferior in its ability to reduce mammographic density and with fewer side effects compared to 20 mg of tamoxifen. Secondly, to study the associations between different doses of tamoxifen and changes in circulating levels of a number of proteins, lipids, hormones, and tamoxifen metabolites, including the impact of polymorphisms in the CYP2D6 gene. A translational aim is to study molecular changes in the healthy breast tissue as a result of tamoxifen exposure.

Statistical Methods: Previous studies have shown that $\sim 50\%$ of women treated with 20mg of tamoxifen have a reduction in density and respond to therapy. We therefore use the *median* density decrease in women treated with 20mg tamoxifen as the predefined response threshold. The primary efficacy endpoint is thus the *proportion of responders* that reaches this threshold. We will test for non-inferiority after treatment with placebo, 1mg, 2.5mg, 5mg, and 10mg compared to the group of women treated with 20mg tamoxifen. Per definition 50% of the women in the 20mg group are responders. The non-inferiority margin is defined to be 16.7 percentage points; that is the fraction of responders is not less than one third of the treated individuals (50% minus 16.7% = 33.3%). The null hypothesis is thus that the proportion of responders in women treated with placebo, 1mg, 2.5mg, 5mg, and 10mg is 33.3% or higher. Power calculations have determined a need of 1200 participants corresponding to 200 participants in each treatment arm.

Present accrual and target accrual: The trial will start recruiting as of September 2016. Eligible women are identified from the Swedish mammographic screening cohort, i.e. women age 40-74 years invited for biennial screening.

Contact information for people with a specific interest in the trial:

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2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-07-01

Title: A randomized trial evaluating bioimpedance spectroscopy vs. tape measurement in the prevention of lymphedema following breast cancer treatment

Ridner S, Shah C, Dietrich M and Vicini F. Vanderbilt University; Cleveland Clinic and Michigan Healthcare Professionals/21st Century Oncology.

Body: Background: Breast cancer related lymphedema (BCRL) represents a common treatment associated complication following surgery, radiation and/or chemotherapy. Increasing data has demonstrated the ability of new diagnostic modalities to detect BCRL in the subclinical phase of the process allowing for early intervention.

Trial Design: This 2-group stratified randomized clinical trial evaluates the effectiveness of bioimpedance spectroscopy (BIS) for early detection and prevention of BCRL compared to tape measurement (TM). Baseline assessments are made pre-operatively. Two-months post-op, patients are censored out if they have developed any of the exclusion conditions, did not have a mastectomy, axillary dissection, >6 sentinel nodes removed, radiation therapy, or taxane. Remaining patients are randomized within site to either BIS or TM; monitored at 3 to 6-month intervals up to 36-months post-op for a change over baseline specified to trigger a compression sleeve & gauntlet intervention. Cohort trigger thresholds are change of ≥ 10 L-Dex units or 5 to $< 10\%$ volume. If the intervention is triggered, measurements by the other method are taken before initiating the 4-week intervention. Post intervention, patients are monitored only with TM. Volume change of $\geq 10\%$ results in study removal and physician referral. At the 2 study endpoints (36 month visit or volume change of $\geq 10\%$) measurements are taken with each method.

Eligibility Criteria: Inclusion criteria: ≥ 18 with histologically confirmed stage I-III breast cancer (BC) or DCIS with planned surgery. Exclusion criteria include history of BC therapy or lymphedema.

Specific Aims: The primary hypothesis is that subclinical detection of BCRL with BIS and early intervention will reduce the rate of lymphedema progression (as measured by referral to complex decongestive physiotherapy) compared to TM. Secondary outcomes include BCRL risk factors, quality of life, and time to treatment.

Statistical Methods: Sample size and powering were based on the hypothesis that BIS would reduce progression rate by 20%. A rate of 50% progression in the TM group was used as the standard. 1100 patients will be enrolled to result in randomized groups of 100 (Total N=200) Statistics include relative risks with respective bootstrapped 95% C.I. and Cochran-Mantel-Haenszel tests.

Present Accrual and Target Accrual: Overall, the study target or expected enrollment as of the end of March 31, 2016 was 690 participants, 534 were actually enrolled (actual accrual 77% of target). Accrual at the study sites ranged from 15 to 104% of target.

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Support: ImpediMed Limited, ImpediMed, Inc. and medi USA.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-07-02

Title: Influence of exercise or educational programs on long-term physical activity by patients after surgery for primary breast cancer: A randomized trial

Kawada K, Taira N, Hatono M, Takahashi Y, Miyoshi Y, Nogami T, Iwamoto T, Motoki T, Sien T, Matsuoka J, Doihara H, Ikeda M, Ogasawara Y, Takabatake D, Yoshitomi S, Kiyoto S, Yamamoto S, Mizota Y and Oka K. Okayama University Hospital, Okayama, Japan; Fukuyama Ci Hospital, Fukuyama, Hiroshima, Japan; Kagawa Prefectural Central Hospital, Takamatsu, Kagawa, Japan; Kochi Health Science Center, Kochi, Japan; Japanese Red Cross Okayama Hospital, Okayama, Japan; Shikoku Cancer Center, Matsumoto, Ehime, Japan; Nashonal Cancer Center Hospital, Chuo-ku, Tokyo, Japan and Faculty of Sport Sciences, Waseda University, Shinjuku-ku, Tokyo, Japan.

Body: [Background]

Past studies revealed that a moderate to high level of physical activity after diagnosis of breast cancer reduces both the risk of breast cancer-related death and death from all causes. Furthermore, some randomized studies suggested that exercise programs improve the percentage of patients who complete the chemotherapy and quality of life, and decrease fatigue, and adverse events. The issues to be determined include defining an established uniform exercise program and the efficacy of a long-term exercise program after breast cancer surgery.

[Object] To elucidate the efficacy of a long-term exercise program and to verify the safety and feasibility of a uniform exercise program using an 'existing social resource' after primary therapy of breast cancer.

[Design] A multi-center, randomized trial.

[Method] Subjects: The subjects included patients who had completed treatment for primary breast cancer, including surgery and/or adjuvant chemotherapy. Patients with metastatic breast cancer were excluded.

Randomization & intervention: The patients were randomly assigned to three groups.

The first group followed an exercise program at Curves® that involved 30 minutes of exercise, including aerobics, weight training, and stretching 3 times a week for 4 months. The second group was given life-style guidance at least once that patients participate in a lecture program about recommended exercise at this point and the importance of weight control after diagnosis of breast cancer using a brochure. The third group served as controls that the patients receive a brochure used same one in the second group. The variables included age and weight.

Outcome: The primary endpoint is level of physical activity at 1 year after randomization, and the secondary endpoints are the percentage of those completing the exercise program, patient reported outcomes (QOL, cancer or treatment associated symptoms, fatigue, depression, and anxiety), body mass index, bone density, and level of lymphedema.

Period of research: The study will last 2 years beginning March 2016.

Sample size: We plan to enroll 400 patients to detect 20% difference with 90% power.

Additional study: Some biochemical markers in the blood will be evaluated to determine the mechanism of the effect of exercise on the human body.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-07-03

Title: Positive behavior change and weight loss in breast cancer survivors on hormonal adjuvant therapy: An energy balance research in cancer (EnBaR) prospective study

Scheuer R, Bendinger GM M, Ensor Jr. JE E, Nixon D, Randolph K, McGuirec E, Rados K, McNight JE E, Pabbathi H, Panicker R, Johnson AT T, Langlois C, Lammersfeld C and Alvarez RH H. Cancer Treatment Centers of America, Newnan, GA and Houston Methodist Research Institute, Houston, TX.

Body: BACKGROUND: Observational studies have repeatedly linked obesity to increased cancer incidence, recurrence, and mortality, leading to cancer treatment guidelines that call for maintenance of a healthy body weight, regular physical activity (PA) regardless of body mass index (BMI), and modest weight loss for overweight and obese cancer survivors (CS). Despite these recommendations, newly published reports suggest that more than 70% of CS are overweight or obese, and only 1/3 engage in the recommended levels of PA. Although trials have demonstrated that energy balance (EB) interventions are feasible in CS, it has been concluded that ongoing behavioral support is needed to implement and sustain changes in weight and PA. Through the subsequent Energy Balance Research (EnBaR) the incorporation of a weight management and PA program focused on ongoing behavioral support for breast cancer survivors (BCS) as an effective method for implementation of lifestyle modifications will be investigated.

TRIAL DESIGN: This is a single-arm prospective observational study investigating if implementation of an EB Program for BCS is an effective intervention for producing lifestyle behaviors. Eligible subjects must be female BCS ≥ 18 years with BMI ≥ 25 who are initiating hormone therapy (HT) as their only oncology treatment modality. Subjects will consult with a Registered Dietitian (RD) to establish goals based on specified interventions for reducing overall BMI at the time of initiating their prescribed HT. Subjects will subsequently receive telephone calls from the RD at bi-monthly intervals to discuss the established points of intervention, answering questions proposed by the RD from a set intervention measurement scale. Patients will also report for body composition analysis performed by the RD via InBody at Baseline, Day 90, and Day 180. InBody is a validated tool that utilizes direct segmental multi-frequency bioelectrical impedance analysis (DSM-BIA) to provide a highly accurate report of an individual's body composition.

AIMS: Determine change in Percent Body Fat (PBF) and BMI during adjuvant hormone treatment for breast cancer patients.

STATS/TARGET ACCRUAL: To assess % change in BMI and PBF between baseline and 180 days, one-sample *t*-tests will be used. Assuming an average (avg) baseline BMI of 33.7 with a standard deviation of 8.5; a sample of 120 patients will yield 89.4% power using a two-sided *t*-test to detect a 13% reduction in BMI to an avg of 29.32 and 82.8% power to detect a 12% reduction to an avg BMI of 29.656. Similarly, a *t*-test will have 83.7% power to detect a 12% decrease in % change in PBF assuming an avg baseline PBF of 41.36 with a standard deviation 10.5. The power calculations were accomplished by generating simulations of size 10,000 replications conducted at the 0.025 significance level using the TTEST procedure of SAS 9.4 [SAS Institute Inc., Cary, NC, USA]. The calculations assume a linear correlation of 0.5 between the baseline and Day 180 measurements and are Bonferroni corrected to maintain a familywise error of 0.05 for the primary analysis. All statistical analyses will be conducted using SAS 9.4. Statistical significance will be defined as $p < 0.05$.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-07-04

Title: A randomized study comparing MoistHer to topical lidocaine for female breast cancer survivors with dyspareunia

Bendinger GM M, Baptist S, Alvarez Tapias C, McGuire E, Rados K, Nixon D, Randolph K, McNight JE E, Pabbathi H, Panicker R, Johnson AT T, Geisler JP P, Manahan KJ J, Lammersfeld C and Avarez RH H. Cancer Treatment Centers of America, Newnan, GA and Instituto Nacional de Cancerologia Bogota, Bogota, Colombia.

Body: BACKGROUND: Problems related to sexual function are known to affect quality of life in female breast cancer survivors (BCS). A leading symptom of sexual dysfunction reported by BCS is dyspareunia, defined as moderate to severe penetrative pain with intercourse that results in reduced frequency of intercourse or abstinence. Dyspareunia in females is most commonly caused by vaginal dryness and primarily treated with estrogen therapy, a contraindication for many BCS. A recent trial found that estrogen-deficient BCS with dyspareunia who applied topical lidocaine to the vulvar vestibule (VV) prior to penetrative intercourse (PI) reported decreased pain during PI and improvement of sexual distress (Goetsch MF, JCO 2015). Identification of additional non-hormonal therapies to target the VV prior to PI may therefore provide treatment options for the improvement of sexual dysfunction in BCS with dyspareunia.

TRIAL DESIGN: This is a double-blind, randomized trial to evaluate the benefits of applying natural lubricant MoistHer (MH) to the VV prior to PI in female BCS with dyspareunia. Subjects must be female ≥ 18 years BCS without active treatment (except for hormone therapy), in a stable heterosexual partnership for ≥ 5 years, and report ≥ 3 months of consistent pain with PI. Patients with a history of pelvic pain, pelvic floor myalgia, vulvar dermatoses, or vaginismus will be excluded. Subjects will be randomized 1:1 to blinded home therapies of either MH or Topical Lidocaine (TL) for application to VV. MH is a commercially available vaginal moisturizer made of emu oil, tocopherol, safflower oil, and aloe extract and will be supplied by manufacturer Dromeo Inc. TL will be a 4% aqueous lidocaine hydrochloride solution prepared by research pharmacist (RP). Both MH and TL will be dispensed by RP in identical unmarked bottles. Subjects will agree to apply study liquid to VV and attempt PI at least twice per week for 4 weeks while maintaining a study diary to record pain scores. Outcomes will be measured via Sexual Function Questionnaire (SFQ), Female Sexual Distress Score-Revised (FSDS-R), and a rating of pain during PI on a scale of zero (no pain) to 10 (worst pain) via Numerical Rating Scale (NRS), to be completed by subjects at baseline, 2 weeks, and 4 weeks.

AIMS: Primary endpoint is pain with PI, to be reported as a score on the NRS. Secondary endpoints are improved quality of sexual life and resumption of PI, to be measured by the SFQ and FSDS-R.

STATS/TARGET ACCRUAL: A total of 50 patients will be recruited and randomized to receive either MH or TL at a 1:1 ratio (25 per group) using the Pocock-Simon dynamic allocation method. This sample size will achieve 94% power to detect at least 1.5 points NRS mean difference between treatments. NRS change will be compared between the two arms using a two-sample t-test (Pooled Standard error or Satterthwaite approximation as appropriate). Secondary outcomes include the SFQ and FSDS-R for which a longitudinal analysis of subscale scores will be conducted using a generalized linear mixed-effects model with fixed effects for treatment group and time. All statistical analyses will be conducted using SAS 9.3 [SAS Institute Inc., Cary, NC, USA]. Statistical significance will be defined as $p < 0.05$.

2016 San Antonio Breast Cancer Symposium

Publication Number: OT3-08-01

Title: A randomized study of personalized music therapy for patients with early stage breast cancer receiving chemotherapy

Toole Jr. M, Bendinger GM M, Ensor Jr. JE E, Alvarez Tapias C, Smith E, McGuire E, Rados K, McNight JE E, Pabbathi H, Panicker R, Johnson AT T, Lammersfeld C and Alvarez RH H. Cancer Treatment Centers of America, Newnan, GA; Houston Methodist Research Institute, Houston, TX and Instituto Nacional de Cancerlogia de Bogota, Bogota, Colombia.

Body: BACKGROUND: A review of the literature identifies high levels of anxiety and depression as adverse effects of oncology diagnosis and treatment, even for patients with curable cancers such as early stage breast cancer (ESBC). Studies have reported that music therapy yields remarkable, multi-dimensional benefits on an individual's mood and state of mind. The purpose of this trial is to evaluate the impact of personalized music therapy (PMT) in reducing anxiety and other adverse mental health symptoms experienced by patients receiving chemotherapy treatment for ESBC.

ELIGIBILITY: Females ≥ 18 years diagnosed with ESBC (Stage I-III) initiating intravenous chemotherapy as their only oncology treatment modality who report anxiety ≥ 4 on a numerical rating scale of 0-10.

TRIAL DESIGN: This is a 4-week, two-arm, randomized (1:1) trial evaluating the anti-anxiety benefits of PMT for women with ESBC initiating intravenous (IV) chemotherapy. The patients randomized to the experimental group will participate in 30-minute PMT sessions conducted by a Licensed Musical Therapist (LMT). Initial PMT will occur within 1 hour of the patient's first chemotherapy infusion (C1D1), then once weekly for the remaining 3 weeks of the trial. Patients randomized to the control group will be referred to the medical oncologist for standard of care (SOC) anxiety treatment. Outcomes will be measured via the Generalized Anxiety Disorder Assessment (GAD-7), the Center for Epidemiologic Studies Depression Scale (CES-D), the Pittsburgh Sleep Quality Index (PSQI), and the Symptom Inventory Tool-M.D. Anderson Symptom Inventory (SIT-MDASI) to be completed by both cohorts at baseline and regular intervals for the duration of the study.

AIMS: The primary endpoint is to determine the impact of PMT during chemotherapy treatment on patient reported anxiety (GAD-7). Secondary endpoints will determine the impact of PMT during chemotherapy treatment on patient reported depression (CES-D), sleep disturbances (PSQI), and quality of life (SIT-MDASI).

STATISTICAL METHODS/TARGET ACCRUAL: Patients will be randomized to receive either PMT or SOC using the Pocock-Simon dynamic allocation method to balance tumor stage between the arms. With 30 patients in each arm and approximate target accrual of 60, the study achieves 80.0% power to detect a 0.65 standard deviation unit effect size of the change in scale measure between baseline and 4 weeks at the 0.05 significance level using a one-sided two-sample t-test. GAD-7 change will be compared between the two arms using a two-sample t-test (Pooled Standard error or Satterthwaite approximation as appropriate). The secondary outcomes include CES-D, PSQI, and SIT-MDASI for which a longitudinal analysis of subscale scores will be conducted using a generalized linear mixed-effects model with fixed effects for treatment group and time. All statistical analyses will be conducted using SAS 9.3 [SAS Institute Inc., Cary, NC, USA]. Statistical significance will be defined as $p < 0.05$.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-01-01

Title: Adding whole-body MRI to body CT scans when evaluating response to systemic anti-cancer therapies alters treatment decisions in metastatic breast cancer

Kosmin M, Makris A, Joshi PV V, Ah-See M-L and Padhani AR R. Mount Vernon Cancer Centre, Northwood, Middlesex, United Kingdom and Paul Strickland Scanner Centre, Northwood, Middlesex, United Kingdom.

Body: Introduction

Accurate evaluation of disease extent and response to systemic anti-cancer therapy (SACT) is key to the clinical management of patients with metastatic breast cancer. By identifying disease distribution and response (particularly progression prior to symptomatic deterioration), imaging aids therapy choices and may maximise quality of life. Whole body MRI (WB-MRI) has increased accuracy for detecting liver and bone disease in breast cancer; however, its potential impact on patient management is largely unexplored. Thus, the purpose of this study was to evaluate the added value of WB-MRI with standard of care CT scans for clinical decision making in routine practice for patients with metastatic breast cancer.

Methods

All patients with metastatic breast cancer who had undergone WB-MRI between 1st April 2009 and 31st March 2016 were screened for this study. Those who had undergone a CT scan of the chest, abdomen and pelvis (CT-CAP) within 14 days of a WB-MRI date were eligible. Original radiology reports for the WB-MRI and CT-CAP were reviewed to establish the extent of reported disease and the SACT response assessment. Contemporaneous medical notes were reviewed to establish the impact of the paired imaging findings (and clinical review) with regard to therapy decisions per time point.

Results

210 pairs of WB-MRI and CT-CAP scans in 101 patients were eligible for analysis. The median age of the studied patients was 56 years (range 23 to 84 years). 46 examination pairs were baseline studies; 164 were undertaken for response assessments (1st line SACT = 46; 2nd line = 27; \geq 3rd line = 58; no information = 33).

In 140 cases (66.7%) there were differences between the extent of disease reported by the WB-MRI and CT-CAP. Of these, 112 (80.0%) were due to the WB-MRI reporting additional sites of disease not evident on CT-CAP, mostly skeletal lesions. CT-CAP showed more disease in 10.0%, mostly lung lesions. 10.0% had some lesions evident only on WB-MRI and other lesions evident only on CT-CAP.

Of the 164 scan pairs performed for SACT response assessment, there were differences in the reported response to therapy in 46 cases (28.0%). 89.1% of disagreements were due to WB-MRI showing evidence of either disease progression (67.4%) or partial response (21.7%) that was reported as stable disease on CT-CAP.

Decisions to change SACT in response to disease progression reported by either/both imaging methods were made in 80 cases. Of these, treatment changes were made due to progression reported only on WB-MRI in 23 (28.8%) cases.

Discussion

This is a retrospective analysis of the real world use of WB-MRI and CT-CAP in the clinical practice of metastatic breast cancer, evaluating their impact on clinical care on a per time point basis. WB-MRI identified additional sites of disease (mostly bone) in over half of patients, which affected SACT decisions in a significant proportion of cases. In many cases, SACT changes would not have been made at the same time point without WB-MRI information. Further research is required to test the hypothesis that earlier identification of disease progression by WB-MRI leads to improved quality of life and patient outcomes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-02-01

Title: Characteristics of physician detected breast cancer

Malmgren JA A, Atwood MK K and Kaplan HG G. HealthStat Consulting Inc, Seattle, WA and Swedish Cancer Institute, Seattle, WA.

Body: Background: Clinical breast examination (CBE) conducted as a part of a routine or annual physical exam is no longer recommended by the American Cancer Society. The U.S. Preventive Services Task Force has not issued any guideline on use of CBE stating there is not enough evidence to recommend for or against the practice.

Methods: A retrospective cohort study was conducted of all physician detected breast cancers (BC) stage 0-IV (N=641) in a dedicated institutional breast cancer registry research database from 1990-2014 (n=12081). Method of detection was chart abstracted at time of diagnosis. Categories are 1) physician or health care professional detection (PhysD) of a palpable lump or abnormality at routine examination prompting a BC work up and diagnosis; 2) patient detection (PtD) of breast symptoms such as a palpable lump, pain, swelling or bleeding which prompted a doctor visit and subsequent BC diagnosis; and 3) mammography detected (MamD) disease discovered by routine mammogram in the absence of complaints or known physical findings. Patients with 'other' or 'missing' diagnosis method were excluded (n=214). Bivariate, multivariate and survival analysis were used to compare characteristics and disease specific survival (DSS).

Results: Over the 25 year time period 5% of breast cancers were PhysD (n=641), 38% PtD (n=4556) and 60% MamD (n=6852). Percentage of PhysD BC decreased from 9% to 3% over time as relative incidence of MamD BC increased with the number per year remaining constant (20-40 cases/year). On average PhysD BC cases were older than PtD BC and younger than MamD BC cases [mean age PtD = 53 years, PhysD = 58 years, MamD = 60 years, $p < .001$] with significantly more PhysD cases age 75 and older [PtD = 8%, PhysD = 15%, MamD = 12%, $p < .001$]. PhysD BC was 56% stage II-IV vs. 23% MamD BC but less than PtD BC (69%) ($p < .001$). PhysD BC was more likely to be hormone receptor negative (15%) and more likely to be triple negative (ER-/PR-/Her2neu-) (11%) than MamD BC (6% for each) ($p < .001$). Mean tumor size of PhysD BC was significantly larger than MamD BC and significantly smaller than PtD BC [MamD BC = 1.69 cm (95% CI = 1.62, 1.76), PhysD BC = 2.55 cm (95% CI = 2.39, 2.70), PtD BC = 3.23 (95% CI = 3.16, 3.31) $p < .001$]. Histology for PhysD BC cases was 7% less ductal and 3% more lobular than either PtD or MamD BC. PhysD BC was less likely to be seen on subsequent mammography (21%) than PtD BC (17%) ($p < .001$). In a multinomial regression model, PhysD BC differed significantly by age, stage and HR status when compared to PtD and MamD BC ($p < .001$). In disease specific survival analysis of the invasive BC cases, the PhysD cases had significantly better DSS than the PtD BC cases but worse DSS than the MamD BC cases [5 year DSS: PtD = 92%, PhysD = 95%, MamD = 98%; 10 year DSS: PtD = 85%, PhysD = 92%, MamD = 95% ($p = .004$ PhysD vs. PtD, $p < .001$ PhysD vs. MamD)].

Conclusions: Clinically detected breast cancer consistently presents as a small but significant portion of the breast cancer population. Physician detected breast cancer is less often seen on subsequent mammogram even after presentation of a palpable mass. While the percentage is modest the cost is minimal. CBE should continue to be taught and practiced by primary care physicians.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-02-02

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-02-03

Title: Evaluating the feasibility of a web-based preference-tolerant randomized trial of risk-based vs. annual breast cancer screening: WISDOM study pilot

Stover Fiscalini A, Theiner S, Kaplan C, Sarrafan S, Sawyer S, Liang A, Rosenberg-Wohl S, Gordon D, Frick M, Borowsky A, Anton-Culver H, Naeim A, LaCroix A, Cink T, Collaboration Athena Breast Health Network and Advocate Partners, Esserman L and van 't Veer L. University of California, San Francisco, San Francisco, CA; University of California, Davis, Sacramento, CA; University of California, Irvine, Irvine, CA; University of California, Los Angeles, Los Angeles, CA; University of California, San Diego, San Diego, CA and Sanford Health, Sioux Falls, SD.

Body: Background: The WISDOM Study (Women Informed to Screen Depending on Measures of risk) aims to examine the effectiveness of personalized breast cancer screening and to bring objective recommendations to the current mammography screening debate. The WISDOM Study is a 100,000 woman randomized trial with a preference-tolerant design that will determine if risk-based screening (RBS) vs. annual screening, is as safe, less morbid, enables prevention and is preferred by women. A pilot was conducted to test the logistics of online participation and examine the acceptance of the study design and approach.

Methods: Women were recruited from the UCSF site of the Athena Breast Health Network, a clinical care-research cohort of 110,000 women from the 5 University of California Medical Centers and Sanford Health. The pilot recruited women via email who were 40 -74 years of age with no history of breast cancer and a normal mammogram in the past year. Those interested visited the WISDOM Study website (wisdomstudy.org), signed up, elected randomization or self-selection, provided electronic consent using DocuSign (eConsent), and completed genetic testing (RBS arm). The Breast Cancer Surveillance Consortium (BCSC) model (standard risk factors, ethnicity, and breast density) in addition to genetic testing (9 genes and 75 SNPs) was used to calculate breast cancer risks that informed the start and frequency of screening for women in the RBS arm. BCSC was also used in the annual screening arm but did not inform mammography screening recommendations. The pilot used a mixed method approach (using enrollment data, Exit Survey data, individual interviews and focus groups) to assess enrollment preferences, randomization acceptance and overall study workflow.

Results: The online electronic enrollment process and patient engagement portal was successfully implemented. In total, 639 women were invited, 235 registered (34%), and 171 (27%) consented to the pilot. Of these, 74% (127) elected to be randomized, and 26% chose to self-assign (66% chose annual screening (29)). Mean age was 56 years and the ethnic breakdown of the cohort was: 79% White, 10% Asian, 7% Latino, 3% Black, 1% other. 92% of those in the risk-based arm of the study completed genetic testing and were given results; only one genetic mutation was identified and occurred in CHEK2. Within the RBS arm (78), mammography recommendations were: 61% no further mammography until the age of 50, 22% biennial, 11% annual, and 6% every 6 month alternating MRI and mammogram. Exit Survey data illuminated confusion in study arm names (risk-based vs. annual), randomization acceptance (74%), annual arm preference in the self-selection group (66%), eConsent satisfaction (90%), enrollment process ease of use (88%), and website content, navigation and appearance satisfaction (66%). The pilot concluded in May 2016 to allow for refinements prior to the full trial.

Conclusion: Our pilot demonstrates that the majority of women are willing to be randomized and participate in an online screening study to answer the important question on optimal breast cancer screening. The pilot study results will inform implementation of the 100,000 women WISDOM Study which launches in fall of 2016.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-02-04

Title: Study on over-diagnosis of high-precision breast cancer screening ~ Consideration from epidemiological point of view

Takebe K and Arai T. Takebe Breast Care Clinic, Takamatsu, Kagawa, Japan.

Body: <Introduction>

In breast cancer screening, it has been attempted to combine US with MMG. For relatively small breasts and dense breasts, there is the opinion that this combination is useful. On the other hand, the problem of over-diagnosis of breast cancer screening has been raised. We've been breast cancer screening with US/MMG combination method since 2005. The results were examined from the epidemiological point of view.

<Subjects and Methods >

Breast cancer discovery rate through US/MMG screening at our facility from 2005 to 2014 was considered. The medical examination was performed on 35,353 women.

Kagawa Prefecture, in which our facility is located has had 100 percent breast cancer registration for more than 10 years. We have compared the breast cancer registration of 2013 with our examination results.

<Results>

Asymptomatic breast cancer detection rate in our breast cancer screening was 0.6%. 65.300%;65.302;% of the detected cancer was DCIS. Low-grade DCIS (Van Nuys 1 and 2) accounted for three-quarters of DCIS. In invasive carcinoma, 10 mm or less of luminal A type was 17%, other luminal types 19%, non-luminal types 5% and unclassified 6%. If our facility's highly precise breast cancer medical examination were performed throughout Kagawa Prefecture, DCIS would be found in 860 cases, 10 mm or less of luminal A type found in 276, other luminal type found in 320 and non-luminal type found in 70 cases per year. Compared to the Kagawa Prefecture breast cancer registered in 2013, DCIS was 6.6 times larger than the registered data, 10 mm or less of luminal A 2.9 times larger, other luminal types 1.1 times larger, and non-luminal types 1.2 times larger. The differences between the registered data and our findings for DCIS and 10mm or less of luminal A type are remarkable.

<Conclusion>

With the US/MMG combination method, the detection rate of DCIS and 10 mm or less of luminal A type have been excessive. Many type of DCIS and tiny luminal A type must exist latently. When performing a high-precision breast cancer screening, it is necessary to note such over-diagnosis. Furthermore, it is also necessary to re-examine the pathology diagnostic criteria of DCIS.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-02-05

Title: Biology and long-term prognosis of screening detected non-palpable breast cancer by ultrasound in hospital-based Chinese population (2001-2014)

Yao R, Pan B, Zhu Q, Xu Q, Zhou Y, Zhang J, Mao F, You S, Lin Y, Shi J, Guan J, Wang X, Zhang Y, Zhang X, Shen S, Zhong Y, Xu Y, Liang Z, Jiang Y and Sun Q. Peking Union Medical College Hospital, Beijing, China; Peking Union Medical College Hospital, Beijing, China and Peking Union Medical College Hospital, Beijing, China.

Body: Background: Milestone studies showed that ultrasound (US) was an effective primary screening test for breast cancer both in the western world and in China [PMID: 26712110, 26715161, and 25668012]. Ultrasound has been officially designated to be the initial imaging test for breast cancer screening in Beijing and several other cities in China, due to its improved sensitivity in Chinese women who usually have denser breasts and develop breast cancer earlier than Caucasian counterparts. Study showed that it would take 40 years to screen each woman in the target age group once [PMID: 26808342]. The mainstay modality of breast cancer screening in China is the hospital-based opportunistic screening among asymptomatic self-referred women. However, there is little data about the tumor biology and long-term survival of the US-detected non-palpable breast cancer (NPBC) in hospital-based Chinese population.

Methods: From January 2001 to December 2014, 3,786 asymptomatic women with positive (BI-RADS 4 and 5) initial screening US underwent biopsies in Peking Union Medical College Hospital, and 572 NPBC in 556 women were diagnosed. Women without dense breasts (defined as BI-RADS category C and D) also received screening mammography (MG) after physical examination and ultrasound. 788 patients with positive (BI-RADS 4 and 5) mammogram (MG) and normal US (BI-RADS 1, 2 and 3) underwent MG-guided biopsies and another 127 NPBC were diagnosed in 126 women. The clinicopathological features, treatment choice, 10-year disease-free survival (DFS) and overall survival (OS) were reviewed and compared between the US-detected and MG-detected NPBC. Prognostic factors of NPBC were identified.

Results: Overall, US could detect more invasive NPBC (83.4% vs 54.3%, $p < 0.001$), lymph node positive cancer (19.1% vs 10.2%, $p < 0.001$) and multifocal cancer (19.2% vs 6.3%, $p < 0.001$). In invasive NPBC, US detected more low grade cancer (21.4% vs 10.2%, $p = 0.001$), multifocal cancer (20.7% vs 2.9%, $p < 0.001$), Her2 negative cancer (77.6% vs 62.3%, $p = 0.001$) and larger tumor ($pT1c+pT2$, 53.3% vs 37.6%, $p < 0.001$). There was no significant difference in immunophenotype/subtype, treatment methods, DFS or OS between US- and MG-NPBC among ductal carcinoma in situ (DCIS), invasive and all NPBC

Table 1. Kaplan-Meier estimates of DFS and OS between US-NPBC and MG-NPBC§.

Patients (No.)		10-year DFS (%)	P value	10-year OS (%)	P value
All	US-NPBC (572)	90.6	0.738	96.1	0.142
	MG-NPBC (127)	92.7		100.0	
DCIS	US-NPBC (94)	100.0	0.060	100.0	-
	MG-NPBC (58)	93.8		100.0	
Invasive	US-NPBC (478)	88.6	0.680	95.2	0.239
	MG-NPBC (69)	92.0		100.0	

§ Kaplan-Meier survival curves between each two subgroups would be displayed in the poster.

. For all NPBC and the US-NPBC, the common DFS-factors included pT, pN and p53 whereas OS-predictors were pN and immunophenotype/subtype.

Conclusion: Compared to MG, US detected more invasive NPBC with positive lymph node in hospital-based asymptomatic self-referred Chinese women, who could achieve comparable 10-year DFS and OS as MG-detected NPBC. US could serve as the feasible initial imaging modality in hospital-based opportunistic screening Chinese women.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-02-06

Title: The impact of the introduction of breast cancer screening on a symptomatic breast cancer unit

Moloney BM M, Malone C, Sweeney K, Barry K, McLoughlin R, Larke A, Walsh S, O Connell AM, Ennis R, McCarthy PA A and Kerin MJ J. Lambe Institute of Translational Research, National University of Ireland, Galway, Connaught, Ireland; National University of Ireland, Galway, Connaught, Ireland and National University of Ireland, Galway, Connaught, Ireland.

Body: Background:

Breast screening with mammography remains the gold standard and is funded in Ireland for women aged 50–64. Several issues including overdiagnosis, overtreatment, false negative mammography, the presence of mammographically occult and interval cancers and has led to much controversy. The symptomatic breast cancer unit deals with all breast cancers and would be expected to be impacted by the National Breast Screening Programme(NBSP), established in 2008.

Aim:

We aim to assess the impact of the introduction of the NBSP on diagnosis of symptomatic invasive breast cancers in the screening population (50 – 64) compared to those 10 years younger (40 – 49) and 10 years older (65 – 75).

Method:

All symptomatic invasive breast cancers between January 2008 & January 2014 were reviewed. Patients were categorised by age (40 – 50, 50 – 64, 65 – 74). Tumour size, grade, subtype and lymph node status were determined. Disease progression was also recorded. Nottingham Prognostic Index (NPI) was calculated for all. Benign disease and non-invasive cancers were excluded.

Results:

A total of 956 patients between 40 and 75 had invasive breast cancer identified between January 2008 and January 2014. 403 of these were between the age of 50 – 64 and eligible for breast screening. A progressive reduction in patient numbers was identified in this age group biennially (Jan 08 – Jan 10; 151, Jan 10 – Jan 12:138, Jan 12 – Jan 14:114). There was no significant change in tumour grade($p=0.775$). Favourable outcomes such as fewer nodal metastasis, a reduction in size, a lower NPI, and less aggressive disease progression were recorded in subsequent biennial groups when compared with the initial group.

Conclusion:

While the outcome may be multifactorial, it is clear that the size, stage and prognosis of the patient group eligible for screening has improved. It can be deduced that this may be attributable to breast cancer screening.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-03-01

Title: An optimized 92-gene assay for the molecular diagnosis of triple-negative breast cancer

Sullivan PS S, Soifer HS S, Liu J, Zhang Y, Schnabel CA A and Brachtel EF F. University of California, Los Angeles, Los Angeles, CA; Biotheranostics, Inc., San Diego, CA and Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Body: Background: Triple negative breast cancer (TNBC) often presents as high grade, poorly differentiated tumors resulting in a more aggressive disease for which accurate and timely diagnosis is critical to treatment selection or clinical trial enrollment. Furthermore, the high rate of distant metastases and absence of breast-specific immunohistochemical markers that contribute to diagnostic uncertainty may delay or limit treatment modalities that can lead to poorer outcomes. The 92-gene assay is an RT-PCR-based cancer classifier that previously demonstrated 80% accuracy for the diagnosis of breast cancer. In this study, blinded validation of an optimized algorithm and assay specifically developed to improve performance in TNBC is described.

Methods: To increase clinical scope for the diagnosis of TNBC, formalin fixed paraffin embedded specimens (N=103) representing a range of breast tumor histologies (e.g. TNBC, adenoid cystic, neuroendocrine, metaplastic, lobular, mucinous, DCIS) were added to the tumor reference database. A revised computational algorithm was constructed by the integration of machine learning techniques. For validation, tumor specimens (N=160) of TNBC (57%) and non-breast tumors (43%) were blindly tested using a 92-gene cancer classifier (CancerTYPE ID®, Biotheranostics, Inc). Tumor type predictions were reported as rank-order probabilities based on the degree of similarity to the tumor reference database. Assay sensitivity based on concordance of the main tumor type prediction with the reference diagnosis established by clinicopathologic review was analyzed.

Results: Assay results included 85 breast carcinomas (TNBC) (53%), 23 Salivary gland carcinomas (14%), and 52 carcinomas (33%) representing 11 other tumor types. For performance in TNBC, the 92-gene assay demonstrated an overall sensitivity of 93% (CI, 86-98), and sensitivities of 96% [95% CI, 89-99] and 80% [95% CI, 52-96], in primary and metastatic tumors, respectively ($P=0.085$). Additional performance characteristics are shown in Table 1.

Table 1

Pathology subset	N, Validation set	N, Correct 92-gene assay predictions	Sensitivity (95% CI)
All TNBC	91	85	0.93 (0.86-0.98)
TNBC-primary	76	73	0.96 (0.89-0.99)
TNBC-metastatic	15	12	0.80 (0.52-0.96)
All Non-breast	69	55	0.80 (0.68-0.88)
Salivary gland carcinoma	25	23	0.92 (0.74-0.99)
Overall performance	160	140	0.88 (0.81-0.92)

Conclusions: An optimized 92-gene assay specifically modified to increase performance for the molecular diagnosis of TNBC showed strong accuracy in this blinded study. These findings support use of the 92-gene cancer classifier to aid in the diagnosis of primary or metastatic TNBC. With more refined tumor characterization, TNBC-specific chemotherapy regimens or clinical trial therapies may be pursued with the potential for improved patient outcomes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-03-02

Title: Breast implant associated anaplastic large cell lymphoma (BIA-ALCL)– The UK experience and first reported case of neoadjuvant brentuximab

Johnson L, O'Donoghue J, Stark H, Collis N, Lennard A, Butterworth M, McLean N, Youssef M, Gui G, Lyburn I, Bristol J, Hurren J, Smith S, Jacklin R, Cunningham D and MacNeill F. The Royal Marsden Hospital, London, England, United Kingdom; Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle Upton Tyne, United Kingdom; Lothian University Hospitals NHS Trust, Edinburgh, United Kingdom; Northumbria Healthcare NHS Foundation Trust, Northumberland, United Kingdom; Gloucestershire Hospitals NHS Foundation Trust, United Kingdom; Portsmouth Hospitals NHS Trust, Portsmouth, United Kingdom and Broomfield Hospital, Chelmsford, United Kingdom.

Body: Introduction The incidence of BIA-ALCL is on the rise. It is a recognised rare risk of breast implants. It commonly presents with a sudden, dramatic seroma around an implant, or occasionally a breast mass. Diagnosis is based on cellular morphology and staining positive for CD30 and negative for anaplastic lymphoma kinase (ALK).

The aetiology and management of BIA-ALCL remains unclear. Clemens *et al.* suggest the conventional Ann-Arbor staging system and aggressive local and systemic treatments may not be appropriate. In the majority of cases, BIA-ALCL is confined to the seroma or the inner aspect of the capsule, so total capsulectomy alone may be sufficient. Systemic therapy may only be required in the minority where disease extends beyond the capsule. We present the UK BIA-ALCL data using the new specific staging system.

Results We report 11 cases of BIA-ALCL from seven regional centres. Treatment was multidisciplinary between breast/plastic surgery and haemato-oncology and based on best available evidence and expert opinion. Mean lead-time from implant to diagnosis was 10 years.

Using the new classification, eight Stage I cases that presented with recurrent seroma were treated successfully with implant removal and total capsulectomy. Four patients had bilateral breast augmentation (BBA); two had bilateral risk-reducing mastectomies (RRM) with implant reconstruction; two had a unilateral mastectomy and implant reconstruction for breast cancer, one of which had previously received adjuvant chemo- radio- and endocrine therapy.

All but one patient with bilateral implants had surgery to remove both implants and ipsilateral total capsulectomy. Of these, three also had contralateral capsulectomy and the pathology was benign. One patient had unilateral capsulectomy and bilateral exchange of implants.

Three patients presented with Stage IIA disease. One had previous RRM and implant reconstruction and presented with a mass. Treatment was CHOP+ (Echelon2 trial), radiotherapy and implant removal, she remains well at two years. One patient with previous BBA following a routine implant exchange developed a BIA-ALCL mass at the drain site. She was treated with local excision, adjuvant CHOP and radiotherapy. She is well at four years.

The third patient had BBA and presented with a mass adjacent to the implant and progressed rapidly through neoadjuvant CHOP to develop life threatening chest wall/thoracic cavity involvement. She achieved complete pathological response with six cycles of Brentuximab followed by bilateral total capsulectomy and implant removal. This is the first reported case of neo-adjuvant antibody therapy in BIA-ALCL.

Discussion Our data support the published literature demonstrating the majority of BIA-ALCL is stage I/II and can be safely managed with surgery alone. Chemotherapy was targeted at patients with more advanced local disease. Brentuximab, a monoclonal antibody is not licenced for BIA-ALCL but is used in refractory in ALCL. BIA-ALCL related death, although rare, is due to uncontrolled local disease progression.

Conclusion BIA-ALCL cases must be staged according to the new system to avoid overtreatment. Brentuximab should be considered first line therapy for locally advanced BIA-ALCL.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-03-03

Title: A blood-based proteomic Videssa® breast assay performs comparably in women with dense and non-dense breasts

Silver M, Tran Q, Gordon K, Benson KL L, Henderson MC C, Letsios E, Mulpuri R and Reese DE E. Provista Diagnostics.

Body: Breast density is associated with reduced imaging sensitivity and specificity for breast cancer (BC). Women with dense breasts are at a four- to six-fold increased risk of developing BC. A biochemical approach that is not affected by density would provide an additional tool to health-care professionals who are managing women with dense breasts and suspicious imaging findings. Videssa® Breast, a combinatorial proteomic biomarker assay, comprised of Serum Protein Biomarkers (SPBs) and Tumor –Associated Autoantibodies (TAAbs) integrated with clinical characteristic data to produce one diagnostic score that reliably detects BC was recently developed as an adjunctive tool to imaging. The goal of this study was to determine whether the diagnostic performance of Videssa® Breast was impacted by breast density.

Provista-001 enrolled 351 participants under the age of 50 years with no prior history of breast biopsy, and Provista-002 cohort one enrolled 210 participants under the age of 50 years with no history of breast biopsy within six months; all participants were assessed as BI-RADS 3 or 4. Breast density status was retrospectively obtained for participants; the four American College of Radiology breast density categories (a, b, c, and d) used for clinical reporting were applied. Serum was collected and tested with Videssa® Breast. Women were categorized into Dense, which included categories c and d, and Non-dense, which included categories a and b, groups.

To understand the performance of Videssa® Breast in women with dense breasts, the clinical sensitivity, specificity, NPV and PPV were evaluated in the dense and non-dense groups from the comprehensive Provista-001 and Provista-002 set (n=545). Of these 545, breast density information was available for 454; 62.6% (n=284) were categorized as having dense breasts and 37.4% (n=170) were categorized as having non-dense breasts. The sensitivity of Videssa® Breast in the non-dense and dense groups was 92.3% and 88.9%, respectively, and the specificity in the non-dense and dense groups was 86.6% and 81.2%, respectively. No significant differences were observed in the sensitivity (p=1.0) or specificity (p=0.1783) of Videssa® Breast in detecting BC in participants with non-dense breasts compared to those with dense breasts. The NPV in both groups exceeded 99%; the PPV was similar across groups.

In summary, this study demonstrates that Videssa® Breast has comparable performance in women with dense and non-dense breasts. Videssa® Breast demonstrates high sensitivity and specificity for detecting BC (Grades I through III), irrespective of density status. Videssa® breast provides an additional tool for health-care providers when women with dense breasts present with challenging imaging findings. In addition, Videssa® breast provides assurance to a woman with dense breasts that she does not have BC, potentially reducing further anxiety in this higher risk patient population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-03-04

Title: To excise or not?: Scoring system for predicting malignancy in patients diagnosed with intraductal papilloma at ultrasound-guided core needle biopsy

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Body: Background: The management of benign intraductal papillomas on core biopsy is controversial. The aim of this study was to determine factors that predict under-evaluation of atypical lesion or malignancy in patients diagnosed with benign papilloma at ultrasound-guided core needle biopsy (CNB), and to develop a prediction algorithm for scoring the possibility of a diagnosis upgrade to atypical lesion or malignancy based on clinical, radiological and pathological factors.

Methods: The study enrolled patients diagnosed with benign papilloma at ultrasound-guided CNB who subsequently underwent surgical excision of the lesion. Multivariate analysis was used to identify relevant clinical, radiological and pathological factors that may predict malignancy.

Results: A total of 520 CNBs led to a diagnosis of benign papilloma (including benign and atypical papillary lesion), of which 452 CNBs were benign papilloma without atypia. Of the 250 lesions in 234 women who underwent subsequent surgical excision, 44 (17.6%) were diagnosed with atypia or malignancy. Multivariate analysis revealed that bloody nipple discharge, size on imaging ≥ 15 mm, BIRADS $\geq 4b$, peripheral location, and a palpable lesion were independent predictors of atypical lesion or malignancy. A scoring system was developed based on logistic regression models and beta coefficients for each variable. The area under the ROC curve was 0.830 (95% CI: 0.665-0.996), and the negative predictive value was 100% for a score ≤ 4 .

Conclusions: A scoring system to predict malignancy in patients diagnosed with benign papilloma at CNB was developed based on five factors: bloody nipple discharge, size on imaging ≥ 15 mm, BIRADS $\geq 4b$, peripheral location, and a palpable lesion. This system was able to identify a subset of patients with lesions likely to be benign, indicating that imaging follow-up rather than surgical excision may be appropriate.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-03-05

Title: Development of a panel of serum-based protein biomarkers for the non-invasive detection of breast cancer in BI-RADS category 4 patients

Chapman KB B, Copeland K, Kidd J, Qiu L, Sheibani N, Tam O, Friedman L, Korn R, Fiorica J, Lourenco A, Suthers S and Hesterberg L. OncoCyte Corporation, Alameda, CA; Boulder Statistics, Boulder, CO; Scottsdale Medical Imaging, Scottsdale, AZ; Sarasota Memorial Hospital, Sarasota, FL; Rhode Island Hospital, Providence, RI and Mercy Clinic Oncology, Oklahoma City, OK.

Body: Background: Current breast cancer screening guidelines call for annual mammography for asymptomatic women age 45 to 54 and once every two years for women age 55 and older. Women with suspicious screening mammograms are recommended for a diagnostic mammogram and may also undergo MRI or ultrasound. Ultimately, suspicious findings unresolved by imaging typically result in the recommendation of a breast biopsy. Approximately 10% of suspicious diagnostic mammograms are recommended for breast biopsies and 67% to 95% of these biopsies yield negative results. With the goal of reducing the number of patients with benign pathology undergoing invasive biopsies, we conducted a screen for serum protein biomarkers and identified a novel panel for the non-invasive detection of breast cancer.

Methods: Serum samples were collected at two sites from women with suspicious diagnostic mammogram findings (primarily BI-RADS category 4) undergoing biopsy for the evaluation of a potential malignancy. Serum samples from 100-patients (50 benign pathology and 50 malignant pathology) were evaluated on the SOMAscan Assay 1.3k, which measures levels of 1,310 different protein analytes. Statistical screening methodologies, such as individual t-tests with control for false discovery, were used to identify markers with the potential to distinguish benign from malignant pathology. The candidate markers were further studied and combined using generalized linear modeling to develop three potential diagnostic models. K-fold cross validation was used to guard against over fitting of the models.

Results: A 15-marker model resulted in an AUC of 0.92 with a sensitivity of 90% and specificity of 76%. Two 6-marker models (with 4 markers in common) each resulted in AUC of 0.85, yielding a sensitivity of 90% with a specificity of 56% or 64%.

Conclusions: This study reveals a novel panel of serum protein biomarkers that may allow for the non-invasive and sensitive detection of breast cancer in BI-RADS category 4 patients. A multicenter study is underway to further refine and validate this panel in a larger set of prospectively collected patient samples.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-03-06

Title: Clinical value of ⁸⁹Zr-trastuzumab PET in HER2-positive breast cancer patients with a clinical dilemma

Schröder CP P, Bensch F, Brouwers AH H, Lub-de Hooge MN N, de Jong JR R, van der Vegt B, Sleijfer S and de Vries EG G. Universital Medical Center Groningen, Groningen, Netherlands; Universital Medical Center Groningen, Groningen, Netherlands; Universital Medical Center Groningen, Groningen, Netherlands; Universital Medical Center Groningen, Groningen, Netherlands and Erasmus MC Cancer Institute, Rotterdam, Netherlands.

Body: Background: Information on human epidermal growth factor receptor 2 (HER2) is essential for management of metastatic breast cancer (mBC). In patients suspected of HER2-positive mBC, standard work up may fail to clarify whole body HER2 status. We aimed to assess whether ⁸⁹Zr-trastuzumab PET can support treatment decisions in patients posing this clinical dilemma.

Methods: ⁸⁹Zr-trastuzumab PET was performed as described earlier (Gaykema et al, Clin Cancer Res 2014) in patients in whom standard work up with bone scan, FDG PET, CT and if feasible a biopsy, failed to evaluate HER2 status of their disease.

⁸⁹Zr-trastuzumab PET was defined positive, when at least a dominant part of the tumor load showed substantial tracer uptake (Gebhart et al, Ann Oncol 2015), when tumor tracer uptake in single lesions (except brain) was \geq normal liver uptake or when brain metastases had a tracer uptake $>$ background. Circulating tumor cell (CTC) analysis prior to tracer injection was performed using the CellSearch System (Janssen Diagnostics LLC) and CTC HER2 status was assessed immunofluorescently.

Questionnaires about treatment decisions were completed before, directly after and ≥ 3 months after ⁸⁹Zr-trastuzumab PET.

Results: Twenty patients were enrolled: 8 with two primary cancers (HER2-positive and HER2-negative BC or BC and non-BC), 7 with metastases inaccessible for biopsy, 4 with prior HER2-positive and HER2-negative metastases, 1 with primary BC with equivocal HER2 status (average 4.23 HER2 gene copies/nucleus). ⁸⁹Zr-trastuzumab PET was positive in 12 patients, negative in 7 and equivocal in one patient. In 15/20 patients ⁸⁹Zr-trastuzumab PET supported treatment decision. The scan altered treatment of 8 patients, increased physicians' confidence without affecting treatment in 10, and improved physicians' understanding of disease in 18 patients. Ten patients had 1-99 CTCs, 6 with HER2 expression. There was no correlation between HER2 expression by CTCs and ⁸⁹Zr-trastuzumab PET results or subsequent treatment decision.

Conclusion: ⁸⁹Zr-trastuzumab PET, but not CTC analysis, supports clinical decision making in BC patients in whom standard work up fails to evaluate HER2 status. (Funded by the Dutch A Sister's Hope).

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-03-07

Title: The measurement of gait speed: An easy way to select older patients with breast cancer for an adapted therapeutic decision making

Pamoukdjian F, Bricou A, Boudabous H, Sebbane G and Zelek L. Avicenne Hospital, HUPSSD, Bobigny, France and Jean Verdier Hospital, HUPSSD, Bondy, France.

Body: Background: Despite recommendations about the adaptation of therapeutic decision making by the International Society of Geriatric Oncology, there is no guidelines concerning breast cancer in older patients for whom a therapeutic decision making should be adapted. Currently, the G8 index $\leq 14/17$ is recommended for patients aged 70 and over before a therapeutic decision making to select patients with a potential frailty. Moreover, gait speed (GS) at the threshold of 1 m/s has been reported in a recent review to identify complications associated with frailty in older outpatients. Indeed, these patients could experience poor outcomes with cancer treatments. For these patients, it is expected that they could not have a standardized decision. We aimed to assess the geriatric profil of older patients with breast cancer for whom a therapeutic decision making was adapted.

Methods: 64 outpatients with breast cancer aged 65 and over were prospectively and consecutively included between November 2013 and April 2016, in two teaching hospitals in a Paris suburb (France). All patients had a G8 index, measurement of gait speed over a short distance (4m) and a multidimensional geriatric assessment before therapeutic decision making. An adapted therapeutic decision making was defined if it not followed treatment recommendations based on tumor characteristics (pTNM, grade, status of hormonal receptors, HER2 status, Ki67 index). A univariate and multivariate analysis by using a logistic regression with a stepwise procedure were performed in all patients with an adapted therapeutic decision. Two models were created to assess the value of G8 index and GS. All variables with $P < 0.20$ were included in both multivariate models. All tests were two sided significant at 0.05. Informed consent was obtained from studied patients prior inclusion. This study was approved by a local committee ethic.

Results: 67% (n=43) of patients had an adapted treatment with a mean age of 82.6 +/- 5.7 years (67-95). Of these patients, 33 had an invasive ductal carcinoma, 23 lymph nod extension, 11 metastatic disease. The mean tumor size was 30.4 +/- 15.4mm. 37 patients had a SBR ≥ 2 , 39 positive hormonal receptors, 5 positive HER2 status. The mean KI67 index was 24.2 +/- 20%. 37 received hormonotherapy, 18 surgery, 16 radiotherapy, 5 chemotherapy. Most of these patients (53.5%) had ECOG-PS ≤ 1 , 39 G8 index ≤ 14 and 36 GS < 1 m/s. In the first model, GS < 1 m/s was the only variable significantly and independently associated with adapted treatment (aOR=5.65, 95%CI: 1.78-19.31, $P=0.003$). In the second model, at least one severe comorbidity (CIRSG grade III, aOR=4.52, 95%CI: 1.30-17.84, $P=0.02$) and cognitive impairment (MMSE $<24/30$, aOR=4.60, 95%CI: 1.32-17.97, $P=0.01$) were the only variables significantly and independently associated with adapted treatment. In the two models, G8 index was not significantly associated with adapted treatment.

Conclusion: A slow gait speed < 1 m/s could be used as a screening test to select older patients with breast cancer for an adapted therapeutic decision making.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-03-08

Title: Use of natural language processing on mammography and pathology findings to supplement BI-RADS to improve clinical decision making in breast cancer care

Puppala M, He TC, Ogunti R and Wong STC TC. Houston Methodist Hospital, Houston, TX.

Body: Breast Imaging Reporting and Data System (BI-RADS) is a standard system used by clinicians to describe mammogram findings and sort the results into categories numbered 0 through 6; this makes accurately communicating about these test results and following up after the tests much easier. For example, the BI-RADS 4 score is given to lesions that carry a risk of malignancy between 2 and 95% such that a majority (69 to 95%) of BI-RADS 4 lesions are biopsied. Our goal is to create a better risk estimate score to stratify patients by developing a more accurate threshold for biopsy.

To accomplish this goal, we defined the personal risk factors, relevant descriptors in the mammography and pathology findings for every patient with BI-RADS 3, 4, or 5 risk score. Clinical data were sourced from the Methodist Environment for Translational and Outcomes Research (METEOR) data warehouse at the Houston Methodist Hospital. METEOR data warehouse contains 135,280 unique patient mammogram reports dating from January 1, 2006 to May 30, 2015. Manual abstraction from this large number of free text reports is not feasible due to the constraints on time and labor costs, and risk of human-error during manual extraction. We developed a natural language processing (NLP) tool called Methodist hOspital Text Teaser (MOTTE) to extract defined clinical parameters and derive the desired meaning from the volumes of free text reports automatically. MOTTE is a rule-based software tool programmed in Java, Structured Query Language (SQL) and an open source machine learning toolkit, OpenNLP. MOTTE combines regular expressions and algorithms to identify where in the dictionary keywords and phrases related to a concept are found.

Mammography findings from January 2006 to May 2015 were extracted including breast density as well as the presence of mass, calcifications, architectural distortion, asymmetric density, and calcification characteristics. A total of 717 patients with BI-RADS 5 mammograms who underwent breast biopsy within 3 months of the abnormal mammogram report date were identified using MOTTE, which also extracted personal history, family history, and subtype information (ER, PR, and HER2 status) of those patients. Clinical data including age, race, height, weight, and body mass index (BMI) were extracted from the METEOR data warehouse for those 717 patients. The extracted data were verified and validated with 99% accuracy by assessing a random 10% of records against a gold standard of manual chart review, which was completed by the clinical coauthors. MOTTE surpasses the manual method in terms of consistency, time, cost, and data preparation. Our ongoing study is to apply the MOTTE to extract distinct characteristics of BI-RADS 4 scored patients. The finding would help to refine biopsy recommendations and create a predictive model to drive evidence-based biopsy decision-making in breast cancer care.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-03-09

Title: A high specificity and sensitivity qPCR Assay for HER2, ER and PR gene expression in breast cancer patients

Rinker KD D, Fuh KF F, McIntyre JB B, Shepherd RD D, Wang H-y, Morris D and Lee H. University of Calgary, Calgary, AB, Canada; Optipharm Inc, Wonju, Gangwon, Republic of Korea; Yonsei University, Wonju, Gangwon, Republic of Korea and Alberta Health Services, Calgary, AB, Canada.

Body: *Introduction:* For breast cancer patients, the reliable assessment of human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER) and progesterone receptor (PR) expression levels is a key determinant for patient stratification and treatment. These markers are currently scored by immunohistochemistry (IHC), with Her2 equivocal samples progressing to in situ hybridization (fluorescent or silver, FISH or SISH, respectively) of DNA amplification. However, FISH/SISH are not quantitative, and may take weeks to deliver an analysis. Further, FISH/SISH do not address the possibility of Her2 gene transcription being an important factor in the Her2 status of a tumour. We developed a novel qPCR assay, the T3 qDx Breast Tumour Profile Test, to enable quantitative assessment of HER2, ER and PR gene expression in a single analysis.

Objective: In this study, we evaluated the performance of the T3 qDx assay in 118 patients in Alberta, Canada, to determine the relationship to a previous 390 patient study conducted in the Republic of Korea. Additionally, the assay was optimized to standardize template input and optimize PCR chemistry for each marker. A comparison of qPCR and digital PCR was made for samples with range of Her2 scores, but focusing on equivocal samples for which the qPCR results disagreed with FISH/SISH. This step was conducted to identify whether transcript levels were independent of either Her2 protein or DNA copies in this sample population.

Materials and Methods: 118 breast cancer FFPE tissue samples obtained between 2005 and 2010 and banked in Calgary, Canada were used in this study. 1mm core punches were made, and RNA was extracted using the RecoverAll™ Total Nucleic Acid Isolation Kit. cDNA was synthesized using the qScript™ cDNA Synthesis kit and stored at -80°C until analysis. HER2, ER and PR expression were quantified using the T3 qDx Breast Tumour Profile Test. qPCR was carried out on a ViiA 7 Real Time PCR Instrument, and relative gene expression calculated using B2M as the reference gene. Digital PCR (dPCR) was performed on a QuantStudio 3D instrument, with copy number determined vs. RNase P. Finally, Her2 test performance was compared to Luminex and an off-the-shelf TaqMan assay for 61 corresponding patient samples.

Results and Discussion: Our results show concordances of 97.2% and 98.1% respectively for unequivocal HER2 and hormone receptor status determined by IHC/FISH (SISH), meeting the minimum concordance threshold of 95% for molecular assays mandated by the American Society of Clinical Oncology/College of American Pathologists. For patients with equivocal HER2 IHC status, the assay showed 86.2% concordance. 75% of the samples with a FISH/SISH score of 2+ were also positive by dPCR. Among the 25% of samples with discordant FISH/SISH and dPCR results, 66% agreed between qPCR and dPCR, while 33% had disparate qPCR and dPCR results. The assay also showed higher specificity and sensitivity than Luminex and an off-the-shelf TaqMan assay for Her2 determination. Taken together, these results indicate that a qPCR approach for determining Her2, ER and PR status can be successful, and that a subset of tumours (~8%) may have transcriptional regulation of Her2 levels that differ from gene amplification findings.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-03-10

Title: Development of a novel HER2 testing strategy, using image-based cell-sorting to isolate pure cell populations from FFPE upstream of FISH

Gerber A, Konig L, Millner L, Strotoman L, Khurana A, Kasimir-Bauer S, Moore MW W, Cotter PD D and Bischoff F. Silicon Biosystems, San Diego, CA; Universitätsklinikum Essen, Essen, Germany; PGXL Laboratories, Louisville, KY and Research DX, Irvine, CA.

Body: Fluorescent in Situ Hybridization (FISH) guidelines defined by American Society of Clinical Oncology (ASCO) and the College of American Pathologists for determining HER2 status are set to improve accuracy and usefulness as a diagnostic marker in breast cancer. Despite these guidelines, many factors can influence HER2 testing results such as sample preparation, assay-conditions and interpretation of test results due to heterogeneous breast cancer samples. In this multi-site study, sample preparation was carried out using the DEPArray™ to recover pure tumor cell populations from formalin-fixed, paraffin-embedded (FFPE) breast tumor samples. We then compared HER2/CEP17 ratios obtained from the DEPArray™ processed samples from each laboratory to routine FISH on tissue sections.

Methods: Eight breast FFPE tumor tissue biopsies were obtained from commercial tissue banks. From the paraffin tissue blocks, four consecutive tissue curls (each 50 microns thick) were prepared. One curl from each of the 8 patient samples was distributed to four different laboratories for analysis following DEPArray™ based sample preparation. After an initial disassociation of each curl into a single-cell suspension, intact cells were sorted and then recovered based on cytokeratin/ vimentin/DAPI staining using the DEPArray™. Cytokeratin+/Vimentin-/DAPI+ tumor (~250) and Cytokeratin-/Vimentin+/DAPI+ stromal (~250) recovered cells were then deposited onto glass slides prior to standard dual-color HER2/CEP17 FISH analysis for comparison to conventional HER2 FISH result.

Results: Serially sectioned breast tumors from 8 negative/positive cases: 7 infiltrating ductal carcinoma (IDC) and 1 metastatic carcinoma were studied. All four sites demonstrated 100% concordance between FISH results compared to the conventional HER2 FISH result. Overall, >60% of DEPArray™ isolated cells were recovered from FFPE samples that ranged from 1- 15 years of age and reported to contain 60% to 80% tumor content. The use of pure sorted cells permitted the accurate determination of HER2 amplification status in only the tumor cells while the stromal cells consistently yielded a more normalized ratio of HER2 to centromere 17.

Conclusion: The preliminary results of this multi-site study demonstrate that use of DEPArray™ for sorted pure populations is reproducible as well as reliable method for subsequent analysis of HER2 by FISH on FFPE derived tumor cells. Given that traditional FFPE-based HER2 FISH results may be influenced by the tissue sectioning procedure, tissue heterogeneity and/or the scattering of few HER2 amplified tumor cells among normal stromal cells. The DEPArray™ allows analysis of immunofluorescence images and DNA content to isolate and recover pure and intact cell populations. This isolation of pure cell populations prior to FISH analysis is attractive for achieving precise determination of HER2 status on equivocal cases. A more formal analytical validation of this approach through CLIA is currently underway.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-04-01

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-04-02

Title: Serum thymidine kinase 1 activity as a pharmacodynamics marker of cyclin-dependent kinase 4/6 inhibition in patients with early stage breast cancer receiving neoadjuvant palbociclib

Liu N, Thomas S, Luo R, Hoog J, Suh EM M, Bergqvist M, Neumüller M, Guo Z, Vij K, Sanati S, Ellis M and Ma C. Washington University School of Medicine, St. Louis, MO; Biovica AB, Uppsala, Sweden and Baylor College of Medicine, Houston, TX.

Body: Background: Thymidine kinase 1 (TK1) is a fundamental enzyme in DNA synthesis. TK1 expression is E2F-dependent and peaks in the S-phase of the cell cycle. In preclinical studies, inhibition of cyclin-dependent kinase (CDK) 4/6 led to dose dependent reduction of TK1 activity in cultured media. We hypothesized that serum TK1 could serve as a non-invasive surrogate marker of cell proliferation in patients (pts) receiving CDK4/6 inhibitors. In this study, we examined serum TK1 activity from breast cancer (BC) pts enrolled on a neoadjuvant study of palbociclib (Palbo) plus anastrozole (A), for changes induced by Palbo, and correlated with changes in tumor Ki67.

Methods: In this phase II neoadjuvant study, 50 pts with clinical stage II or III estrogen receptor positive (ER+) HER2- BC, received A (in combination with goserelin if premenopausal) alone for 28 days in cycle 0 (C0), followed by the addition of Palbo (125 mg daily on days 1-21) on cycle 1 day 1 (C1D1) for 4 28-day cycles (C1 to C4) unless C1D15 tumor Ki67>10%, in which case pts went off study. Following completion of cycle 4, A was continued for another 3-5 weeks to allow Palbo washout prior to surgery, except in 8 pts who received an additional 10-12 days of Palbo immediately prior. Blood and tumor biopsies were collected at 4 time points: baseline, C1D1, C1D15, and surgery. Serum TK1 activity was measured using the highly sensitive Divitum™ assay according to the Divitum™ Instructions for use (Biovica, Sweden). Tumor Ki67 IHC was performed at the Washington University AMP laboratory using the CONFIRM anti-Ki67 rabbit monoclonal antibody (clone 30-9), and pathologist guided image analysis.

Results: There was no statistically significant difference in TK activity between baseline and C1D1 serum samples (Table 1). However, serum TK activity decreased significantly from C1D1 to C1D15 following the addition of Palbo and increased significantly from C1D15 to surgery following Palbo washout (Table 1), indicating a significant effect of Palbo on TK activity. At C1D15, TK activity was below the detection limit of 20 Du/L in 44 of 48 pts, and was at low levels (24, 26, 26, and 58 Du/L) in the remaining 4 pts, indicating a profound effect by Palbo. Interestingly, the TK activities of the 4 pts with tumor Ki67 >10% at C1D15 were all below 20 Du/L, suggesting the possibility of tumor cell proliferation independent of CDK4/6 inhibition. The sensitivity and specificity of change (increase/decrease) in serum TK activity to predict tumor Ki67 (increase/decrease) induced by Palbo were 83% (19/23, 95%CI: 66-99%) and 93% (26/28, 95%CI: 83%-100%), respectively. The Kappa statistic was 0.761 (P<0.001), indicating substantial agreement between the two tests.

Conclusions: Serum TK1 activity may serve as a pharmacodynamics marker of CDK4/6 inhibition and further investigation is warranted.

Table 1. Serum TK1 and tumor Ki67

	Serum TK		Ki67	
	Median (IQR) (Du/L)	N	Median (IQR) (%)	N
Baseline	46 (25-73)	48	24.34% (11.92%-35.43%)	45
Cycle 1 day 1	43 (27.5-98)	49	5.37% (2.49%-13.59%)*	45
Cycle 1 day 15	20 (20-20)*	48	0.78% (0.23%-1.05%)*	45
Day of surgery	136.0 (37.5-259)*	37	8.33% (2.25%-23.03%)*	34

*P<0.001 compared to the preceding time point.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-04-03

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-04-04

Title: Activation of ER β in triple negative breast cancer results in cell cycle arrest

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Body: Background: Triple negative breast cancer (TNBC), which comprises approximately 20% of breast cancer diagnoses, lacks estrogen receptor alpha, progesterone receptor and Her-2 expression. However, we have identified that 30% of TNBC patients express estrogen receptor beta (ER β), a nuclear hormone receptor and potential therapeutic target. Here we examine the effects of ER β in triple negative breast cancer cell lines.

Methods: Cell lines that stably express ER β were used to perform microarray analyses following five days of estrogen treatment. Ingenuity pathway analysis was conducted on differentially expressed genes to determine alterations in biological pathways. The effects of ER β on cell cycle progression and apoptosis was determined. Cell cycle-related expression changes were confirmed with RT-qPCR and western blotting. The impact of siRNA mediated gene silencing of CDK1 on TNBC cell proliferation was assessed as was the ability of ER β to elicit anti-proliferative effects in the setting of CDK1 knockdown.

Results: We have shown that estrogen or ER β -specific agonist treatment causes decreased proliferation of ER β + TNBC cells. This inhibitory effect is not due to programmed cell death but rather a G1/S phase cell cycle arrest as indicated by flow cytometry experiments. Microarray data and ingenuity pathway analysis revealed a number of down regulated genes involved in cell-cycle progression. Specifically, estrogen treatment of ER β positive TNBC cells was shown to result in suppression of cyclin-dependent kinase 1 (CDK1) and Cyclin B, effects that were confirmed following ER β -specific agonist treatment at both the mRNA and protein levels via RT-qPCR and western blotting, respectively. Knockdown of CDK1 in ER β + TNBC cells using siRNA resulted in decreased proliferation and diminished the anti-proliferative effects observed following estrogen or ER β -specific agonist treatment.

Conclusions: Our data demonstrate that estrogen and ER β -specific agonists cause cell cycle arrest in ER β positive TNBC. These effects are due to ER β -mediated suppression of multiple genes involved in cell cycle progression including CDK1 and Cyclin B. Following knockdown of CDK1, estrogen or ER β -specific agonist treatment displayed minimal impact on cell proliferation. Therefore, ER β 's effects on proliferation may primarily be mediated by blockade of CDK1 and Cyclin B. Regardless of ER β , our data suggest that inhibition of CDK1 activity may have therapeutic benefit in a subset of TNBC patients, an area of study that has yet to be explored.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-05-01

Title: Physico-biochemical regulation of EMT by microtubule associated protein 7 (MAP7)

Choi DS, Dave B, Rosato RR R and Chang JC C. Houston Methodist Cancer Center, Houston, TX.

Body: Background: We previously reported about 500 cancer stem cell (CSC) specific gene signatures from patient tumor samples. After screening with shRNAs for the 500 genes affecting mammosphere forming ability, we identified microtubule-associated protein 7 (MAP7) as one of the top candidate genes, which may serve as a target for breast CSCs. Although MAP7 is a predominant epithelial microtubule binding protein, only limited number of reports suggests that MAP7 may be a regulator of microtubule dynamics during cell division and a cofactor of Kinesin-1 in compartment transport in cells. However, little is known about how MAP7 supports epithelial cancers, especially breast cancer. Previously, we have reported that the mammosphere forming cells exhibit treatment resistance and high metastatic potential, which are intrinsic characters for CSCs displaying epithelial mesenchymal transition (EMT). We hypothesize that MAP7 supports breast cancer progression by promoting CSC self-renewal and survival through regulation of EMT.

Objectives: Here, we aim to show that MAP7 is an essential regulator of breast CSCs and to elucidate mechanism behind EMT regulation by MAP7 in breast CSCs.

Methods and Results: On Oncomine database analysis, MAP7 was up-regulated in most epithelial cancers, when compared to the corresponding normal tissues. Similarly, its expression in breast cancer was 2-fold higher than in the normal breast tissue ($p < 0.05$), but without significant variances in the expression across the breast cancer subtypes. Gene silencing of MAP7 significantly reduced CD44⁺/CD24⁻ breast CSC populations and mammosphere forming efficiencies of MDA-MB-231, HCC1937, and MDA-MB-468 breast cancer cells. Furthermore, the silencing of MAP7 expression compromised invasive potential of MDA-MB-231 cells by 50% and significantly altered the cell membrane mechanics of MDA-MB-468 cells, as indicated by a high-content image analysis for cell shapes and cell adhesion efficiency. More importantly, delivery of siRNA in vivo inhibited the growth of BCM2147 patient-derived tumor, and limiting dilution assay demonstrated that the tumor initiation potential of BCM2147 can be eliminated by MAP7 silencing. Through confocal microscope analysis of images of fluorescent immunostaining and co-immunoprecipitation assays, MAP7 showed polarized-expressions in spindle-shaped cancer cells and was co-localized with Focal Adhesion Kinase (FAK). Moreover, MAP7 silencing inhibited the phosphorylation of FAK by inactivating p130CAS and JSAP1, the upper stream and the down-stream regulators of FAK.

Conclusion: We have showed the ectopic expression of MAP7 in breast tumors and other epithelial tumors, suggesting MAP7 may be involved in tumorigenesis and critical for the survival of tumor cells. Moreover, our results suggest that MAP7 is a key element for survival and self-renewal of breast CSCs through polarization of cells and activation of FAK, required for the initiation of EMT. To that end, here we report that MAP7 is essential for breast cancer growth by supporting CSC survival and self-renewal.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-05-02

Title: NEDD9 promotes breast cancer metastasis by regulating mitochondrial functions

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Body: NEDD9 has been characterized as a metastasis-promoting gene in various cancer cells including breast. We previously reported that NEDD9 promotes malignant phenotypes of breast cancer cells through distinct and non-overlapped domains. For example, the FAT (Focal Adhesion Targeting) domain of NEDD9 promotes cancer cell growth, while the SH-domain facilitates cell migration. These results suggest that NEDD9 promotes tumor metastasis by enhancing dissemination and growth in the tumor-host microenvironments through distinct and non-overlapped domains. Thus, targeting functions of NEDD9 is a promising approach for breast cancer therapies.

In order to further characterize NEDD9-mediated breast cancer growth, we performed yeast-two hybrid (Y2H) screening to identify proteins that associate with the FAT domain of NEDD9. Using the FAT domain constructed in pGBKKT7 (Clontech, CA) as a bait to screen library of human fibroblast (Clontech, CA), we identified several proteins that associate with the domain. They are small GTPases (i.e. RAB11a and ARF4), cytoskeletal proteins (i.e. Nexilin), and cytosolic proteins (i.e. HAX-1). Among of these potential partner proteins, we focused on the interaction between NEDD9 and HAX-1 in breast cancer cells.

Co-immunoprecipitation assays confirm the molecular complex of NEDD9-HAX-1 in both SK-Br3 and SUM149 cells. Importantly, p130cas, which harbors similar domain structures with NEDD9, was not precipitated with NEDD9, suggesting a specific interaction between NEDD9 and HAX-1. Given the fact of NEDD9 as a key metastasis promoting gene, these results suggest that NEDD9-HAX-1 plays a key role breast cancer metastasis by facilitating growth in microenvironments.

While the biological function are not clear at present, previous studies demonstrated that HAX-1 localizes in mitochondria in breast cancer cells, Indeed, we demonstrated that NEDD9 was found in both cytosol and mitochondria fractions in malignant breast cancer cell MDA-MB-231, but not non-metastatic HCC38. These results suggest the presence of NEDD9-HAX1 complex in mitochondria and this complex may facilitate breast cancer metastasis. In addition to HAX-1, several mitochondrial proteins such as EFG1, DCTN6, and MMADHC were found in the Y2H screening system as described above. These results suggest that NEDD9 facilitates breast cancer metastasis through regulating multiple pathways including signaling pathways and mitochondrial functions, thus serving as a promising therapeutic target for cancer patients including breast.

The view expressed in this article are those of the author and do not reflect the official policy of the Department of Defense, or U.S.Government.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-05-03

Title: Upregulated purinergic signaling enhances cell proliferation in human and murine breast carcinomas

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Body: The composition of the extracellular tumor microenvironment differs from that of most other tissues and is thought to provide cancer cells with a growth and survival advantage compared to normal cells. In solid tumors, the extracellular concentration of ATP can be elevated to ~100 μM and extracellular pH can be as low as 6.5. In the current project, we investigate the consequences of purinergic signaling in human and murine breast carcinomas: we study intracellular Ca^{2+} signals and associated changes in cell proliferation during stimulation with extracellular nucleotides.

We employ biopsies of human and murine primary breast carcinomas and compare them with matched normal breast tissue.

Human biopsies are obtained with written informed consent from patients undergoing breast conserving surgery at Aarhus University Hospital or Regional Hospital Randers in Denmark. Murine biopsies are from mice overexpressing unactivated ErbB2 specifically in the breast tissue. We isolate epithelial organoids (~150 μm diameter) from tissue biopsies by partial digestion with collagenase III. Organoids loaded with the Ca^{2+} -sensitive fluorophore Fura-2 are studied by fluorescence microscopy. In separate experiments, cell proliferation is quantified by detecting newly synthesized DNA using immunofluorescence imaging of organoids incubated with the thymidine analogue bromodeoxyuridine (BrdU).

We find that intracellular Ca^{2+} responses during stimulation with extracellular ATP are elevated 2- to 10-fold in breast carcinomas from mice and humans, respectively, compared to matched normal breast tissue. We observe similar differences between breast cancer tissue and normal breast tissue in response to stimulation with the $\text{P2Y}_2/\text{P2Y}_4$ -agonist UTP, whereas virtually no rise in the intracellular concentration of Ca^{2+} is observed in response to the P2X7 -agonist 3'-O-(4-benzoyl)benzoyl-ATP. Application of cyclopiazonic acid – an inhibitor of the sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase – also cause exaggerated intracellular Ca^{2+} responses in breast cancer compared to normal breast tissue. Consistent with the elevated Ca^{2+} responses, stimulation with 100 μM ATP or 100 μM UTP increases the rate of cell proliferation (i.e., fraction of BrdU-positive cells) by ~2-fold in the breast cancer tissue.

In conclusion, we find that purinergic signaling is upregulated in human and murine breast carcinomas compared to normal breast tissue. Activation of purinergic receptors – most likely P2Y_2 and/or P2Y_4 – enhances cell proliferation in breast cancer tissue. We propose that the high ATP levels in the tumor microenvironment promote breast cancer development or progression and that the associated signaling pathways represent promising targets for therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-05-04

Title: Vitamin K dependent gamma-carboxylation in breast cancer

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Body: The secreted protein periostin (PN) is strongly linked to breast cancer aggressiveness and serum PN is a promising marker of disease activity. However, the regulation and function of PN in relation to the cancer phenotype has yet to be clearly defined. PN is one of the rare proteins (<15 in mammalian systems) that is subject to the post-translational addition of γ -carboxylated glutamatic acid (GLA) residues. However, no studies have addressed whether γ -carboxylation of PN has functional relevance in breast cancer, despite evidence that the proteins that catalyze γ -carboxylation are expressed in normal mammary gland. Using publically available datasets we found that GGCX, VKORC1 and VKORC1L1 (genes that mediate γ -carboxylation) are overexpressed in 24% of breast cancers. Furthermore, survival of patients whose tumors overexpress these genes is significantly worse than that of patients whose tumors do not overexpress these genes. Follow-up studies utilized the Human Protein Atlas to examine expression of GGCX, VKORC1 and VKORC1L1 proteins in normal breast and invasive ductal carcinomas (IDC). Normal ductal epithelial cells showed intense staining for GGCX, patchy moderate staining for VKORC1 and diffuse low signal for VKORC1L1. All cases of IDC exhibited strong staining for GGCX, 80% had moderate-intense staining for VKORC1 and 77% had diffuse but low staining for VKORC1L1. Staining for all 3 proteins was localized only in tumor cells indicating that stromal cells are unlikely to contribute to protein γ -carboxylation. Next we explored expression of the γ -carboxylation pathway genes using *in vitro* models of epithelial-mesenchymal transition (EMT) and tumor progression. The EMT model consists of human mammary epithelial cells immortalized with telomerase and SV40 (HMLE cells) that constitutively express GFP (control), TWIST, SNAIL or TGF β . Relative to control cells, GGCX, VKORC1 and VKORC1L1 were up-regulated in HMLE-TWIST cells, GGCX and VKORC1 were up-regulated in HMLE-SNAIL cells and VKORC1 was up-regulated in HMLE-TGF β cells. These data suggest that γ -carboxylation may be triggered early in breast cancer oncogenesis. In the tumor progression model, we found up-regulation of GGCX and VKORC1 in HMLE+RAS cells relative to HMLE cells. Introduction of SV40 alone did not alter expression of these genes. In HMLE-TWIST and HMLE-RAS cells with the highest expression of GGCX, VKORC1 and VKORC1L1, PN was up-regulated 200-300-fold, supporting the concept that PN may be a relevant target for γ -carboxylation during EMT and tumor progression. In summary, available genomic and proteomic data suggest that the vitamin K dependent pathway genes GGCX, VKORC1 and VKORC1L1 are present in normal mammary gland but up-regulated in a subset of invasive breast cancers that are characterized by poor overall survival. Genes in this pathway are also up-regulated in mammary epithelial cells expressing triggers of EMT (particularly TWIST) or oncogenes such as RAS. Since the only known function of these genes is in γ -carboxylation, studies to confirm PN as a GGCX substrate and to identify additional γ -carboxylated proteins and their functions in breast cancer are clearly warranted. Our limited understanding of this ancient, conserved pathway may be masking an important therapeutic opportunity.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-05-05

Title: The role and regulation mechanism of HOXB5 in human breast cancer cells

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Body: HOX genes are transcription factors that play important roles in body patterning and cell fate specification during normal development. Among of these, HOXB5, is involved in a variety of developmental processes, particularly during the enteric nervous system (ENS) development, and thus, abnormalities in HOXB5 function during embryo stages lead to Hirschsprung's disease. Importantly, many HOX genes, including HOXB5, are expressed not only during embryogenesis but also in adults and are dysregulated in various cancers. In a previous study, we found aberrant overexpression of HOXB5 in breast cancer tissues and cell lines and demonstrated that HOXB5 is important in the regulation of cell proliferation in breast cancer cells. Also, HOXB5 induces invasive potential through epithelial-mesenchymal transition (EMT). The relationship between HOXB5 and phenotypic changes in MCF7 breast cancer cells has been studied, however, HOXB5 functions as a transcription factor and its involvement in signaling pathways remain unclear. In this study, we selected putative downstream target genes of HOXB5, such as interleukin (IL)-6, Snail2 and epidermal growth factor receptor (EGFR) by PCR array analysis. These genes have been reported to be involved in cancer progression, which is characterised by increased growth speed and invasiveness of the tumor cells. Here, we discovered that HOXB5 transcriptionally upregulates the promoter activity of these genes. Chromatin immunoprecipitation (ChIP) analysis to confirm direct binding of HOXB5 to the promoter region is now ongoing. Since we found that HOXB5 induces EGFR protein expression and SRC phosphorylation, we will further investigate signaling pathway components to understand the underlying molecular mechanisms of HOXB5 action in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-06-01

Title: Talazoparib antitumor effects in *BRCA*-deficient breast cancer models

Phan VT T, Protter AA A, Peterson A and Uppal H. Medivation, Inc., San Francisco, CA.

Body: Background: *BRCA1* and *BRCA2* functions are essential for the DNA double-strand break repair process in living cells with DNA damages. Therefore, germline pathogenic mutations in *BRCA1/2* increases the risk of developing cancer.¹

²Poly(ADP-ribose) polymerase (PARP) enzymes are proteins responsible for DNA single-strand break repair. Persistent inhibition of PARP-dependent DNA repair in *BRCA*-deficient breast cancer cells leads to increased DNA damages resulting in cancer cell death. Talazoparib is a novel and potent, orally bioavailable, small molecule PARP inhibitor. Talazoparib's dual mechanism of action inhibits PARP enzyme activity and effectively traps PARP on DNA, preventing DNA repair, resulting in cell death in *BRCA1/2*-mutated cells.³ In tissue culture studies, talazoparib is more potent at trapping PARP on DNA to induce cancer cell death compared to other PARP inhibitors.⁴ Previous studies have demonstrated talazoparib inhibited growth in tumors harboring *BRCA1/2* gene mutations. In the MX-1 breast cancer model with *BRCA1*-deficiency, talazoparib inhibited cell growth in vitro and induced regression in solid mouse xenografts.⁵ Here, we demonstrate antitumor effects of talazoparib monotherapy in a panel of breast cancer cells and patient-derived breast cancer models with pathologic *BRCA1/2* mutations.

Method: A panel of human breast cancer cell lines was treated with talazoparib to determine its cytotoxic effects. *BRCA1/2* mutations status was correlated to talazoparib cytotoxic effects. At the molecular level, *BRCA1/2*-mutant and wild type breast cancer cell lines were treated with dose-escalating talazoparib to also assess the relationship between PARP-DNA trapping complex formation and treatment response. Patient-derived breast cancer xenograft models were used to assess talazoparib monotherapy on tumorigenesis. Immunohistochemistry assays were performed to determine Ki-67, γH2AX and caspase 3 marker expression following talazoparib treatment.

Results: Cytotoxicity was observed in 50% (7/14) cell lines at IC₅₀ values that are achieved in the clinic. *BRCA1/2* alterations were detected in 21.4% (3/14) of cell lines sensitive to talazoparib treatment. In 14 patient-derived breast cancer xenograft models selected for this study, 57.1% (8/14) responded to talazoparib monotherapy. Of these, 35.7% had mutations in the *BRCA1* (28.6%) and *BRCA2* (7.1%) genes. Stable disease was observed in 14.2% (2/14). One stable model had *BRCA1* mutations. Tumor regression was observed in 42.8% (6/14) of the models treated with talazoparib monotherapy. Importantly, 66.7% (4/6) of patient-derived breast cancer models that regressed on talazoparib monotherapy had mutations in *BRCA1/2* genes.

Conclusions: Cytotoxicity was observed with talazoparib monotherapy in breast cancer cell lines and in patient-derived xenograft tumor models harboring *BRCA1* or *BRCA2* mutations. Our data demonstrate therapeutic potential of talazoparib monotherapy in breast cancer associated with pathologic *BRCA1/2* mutations.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P5-06-02

Title: Androgen receptor signaling regulates survival to ionizing radiation in breast cancers

Yard BD D and Abazeed ME E. Cleveland Clinic Taussig Cancer Institute, Cleveland, OH.

Body: Background: Radiotherapy administered following breast-conserving surgery significantly reduces the risk of local recurrence and breast cancer mortality. However, a key limitation to radiotherapy has been the lack of biomarkers that reliably predict response to treatments. There is an urgent need to identify patients with tumors that are resistant to radiotherapy and to improve therapeutic efficacy in these patients. To advance genotype-directed radiotherapy in breast cancer, we identified determinants of survival by leveraging genomic data with a novel and validated high-throughput platform for measuring radiation sensitivity and discovered an association between androgen receptor (AR) expression and resistance to radiotherapy.

Methods: We profiled 28 breast cancer cell lines using our recently validated high-throughput assay to measure survival. Survival curve analyses permitted quantitative assessment of radiosensitivity. Gene Set Enrichment Analysis (GSEA) was used to correlate radiosensitivity with genomic features of the profiled cell lines. Cell growth and death were measured by CellTiter-Glo® and colony formation assays, respectively. DNA damage was measured by neutral comet assays. γ -H2AX was measured by Western immunoblot. Mouse xenografts were prepared by the orthotopic injection into the mammary gland of female NOD *scid* gamma mice.

Results: GSEA nominated several pathways that are known to confer resistance to DNA damage. However, some of the most correlated and intriguing gene sets were associated with AR signaling. We showed that AR mRNA levels were directly correlated with radiation survival (Pearson's $r=0.48$). To test the interaction between androgen signaling and radiation survival, we employed the AR-positive triple-negative breast cancer cell line MDAMB453. Re-supplementation of steroid deprived media with dihydrotestosterone (DHT) prior to radiation resulted in a dose dependent rescue of cell growth, while treatment with the potent AR antagonist enzalutamide sensitized the cells to radiation. Similar results were observed for other AR-positive cell lines (HCC202, ZR7530, CAMA1, and BT474) independent of ER and ERBB2 status. To assess whether androgen ablation cooperates with ionizing radiation *in-vivo*, MDAMB453 xenografts were exposed to either enzalutamide, radiation, or the combination of enzalutamide and radiation. The combination of enzalutamide (15mg kg⁻¹ or 25mg kg⁻¹) and ionizing radiation suppressed tumor growth more potently than either enzalutamide or radiation alone. Using neutral comet assays immediately following radiation exposure, we determined that incubation of MDAMB453 and BT474 cells with enzalutamide resulted in an increased number of double-strand breaks while treatment with DHT reduced DNA damage. Additionally, γ -H2AX levels remained elevated in enzalutamide treated cells following radiation treatment. Taken together, these data indicate that AR signaling protects breast cancer cells from DNA damage.

Conclusion: Integration of high-throughput radiation survival profiling with large-scale cancer genomic data indicates that androgen signaling is associated with radiation resistance in breast cancers. Radiotherapy of AR-positive breast cancers may be improved by neoadjuvant and concomitant anti-androgen therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-06-03

Title: A solution to the APOBEC mutation paradox in breast cancer

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Body: Breast cancer has a major genetic component, and individual tumors can have thousands to tens-of-thousands of mutations. Large proportions are cytosine mutations in TCA and TCT trinucleotide motifs. A variety of studies have attributed this mutation signature to the APOBEC family of DNA cytosine deaminases, with previous literature favoring APOBEC3B as the most likely enzyme to be responsible (from a total of nine active family members). However, breast tumor genomic DNA sequences from patients with a naturally occurring germline deletion of the entire APOBEC3B gene still show an intact APOBEC mutation signature.

To resolve this paradox, we tested the hypothesis that the only other functionally dimorphic APOBEC family member, APOBEC3H, may be responsible. First, we performed an unbiased genetic analysis of TCGA data sets and showed that APOBEC3B-null tumors with this mutational bias have at least one copy of the haplotype-I variant of APOBEC3H, despite weak genetic linkage between these two genes ($n=14/14$). Remarkably, breast tumors without APOBEC3B and APOBEC3H haplotype-I showed no evidence for an APOBEC mutation signature ($n=3/3$). The proportion of APOBEC signature mutations between the cohort with and the cohort without APOBEC3H haplotype-I was highly significant ($p<0.00001$ and lower), indicating that these two enzymes alone may be fully responsible (and casting serious doubt on the potential role of APOBEC3A). Second, although deemed inactive in prior studies, we discovered that APOBEC3H haplotype-I has robust activity in biochemical assays with mutation of TCA-containing single-stranded DNA and in cellular assays with hypermutation of HIV-1 cDNA and mutation of a genomic DNA eGFP reporter gene. Third, the subcellular localization APOBEC3H haplotype-I and other expressed variants of this enzyme differed with only the latter showing significant nuclear localization in a panel of cell lines. The genomic mutagenicity and localization of APOBEC3H haplotype-I is attributable to a glycine at position 105, whereas other variants of this enzyme that have an arginine at this position, suggesting a general molecular mechanism. These studies combine to offer a parsimonious solution to the APOBEC mutation paradox with APOBEC3B and APOBEC3H haplotype-I, together, accounting for the strong "APOBEC mutation signature" observed in many breast cancers.

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Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-06-05

Title: Preclinical characterization of VX-984, a selective DNA-dependent protein kinase (DNA-PK) inhibitor in combination with doxorubicin in breast and ovarian cancers

Boucher D, Newsome D, Takemoto D, Hillier S, Wang Y, Arimoto R, Maxwell J, Charifson P, Fields SZ Z, Tanner K and Penney MS S. Vertex Pharmaceuticals Incorporated, Boston, MA.

Body: Background: The efficacy of chemotherapeutic agents such as doxorubicin, which cause lethal DNA double-strand breaks (DSBs), is diminished by efficient repair of the damaged DNA in cancer cells. DNA-PK is a key regulator of the non-homologous end joining (NHEJ) pathway, which is responsible for repairing DSBs. Studies of nonselective inhibitors of DNA-PK have shown that cancer cells depend on DNA-PK for survival following treatment with DSB-inducing agents. However, a comprehensive characterization of DNA-PK inhibition has been hampered by a lack of selective inhibitors. Here we describe VX-984, a potent and selective inhibitor of DNA-PK, and its preclinical profile in combination with doxorubicin both in vitro and in vivo.

Methods: VX-984 was examined as a single agent and in combination with doxorubicin or pegylated liposomal doxorubicin (PLD) in a panel of breast cancer cell lines and in mouse xenograft models, respectively.

Results: In vitro, inhibition of DNA-PK by VX-984 enhanced the cytotoxic activity of doxorubicin in established breast cancer cell lines and in primary ovarian tumor explants. Notably, mean Bliss DE >10% (strong synergy) were observed for doxorubicin in the presence of VX-984 in 22 of 35 breast cancer cell lines and 21 of 44 ovarian cancer cell lines in a broad cancer cell line screen. Further, the efficacy observed with VX-984 was associated with increased DNA damage as measured by phosphorylated histone H2AX (gamma-H2AX) and phosphorylated Kruppel-associated protein (pKAP1) in DU4475, MDA-MB-436 and MDA-MB-468 breast cancer cell lines, which is consistent with diminished DSB repair. In vivo, VX-984 significantly enhanced the efficacy of PLD in ovarian cancer patient-derived xenograft models and in cell line xenograft models.

Conclusions: These data provide evidence that inhibition of DNA-PK by VX-984 enhances the efficacy of doxorubicin in preclinical models and support the use of VX-984 in combination with DSB agents such as anthracyclines including PLD for the treatment of breast and ovarian cancers. VX-984 is currently in a Phase 1 clinical trial in combination with PLD. Sponsored by Vertex Pharmaceuticals Incorporated.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-06-06

Title: Comprehensive analysis of the DNA damage repair and maintenance pathways that regulate TNBC sensitivity to replication stress

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Body: Agents that induce replication stress, such as inhibitors of Chk1 or ATR, are advancing in clinical development and are being tested for treatment of various solid tumors, including triple-negative breast cancer (TNBC). While the preclinical data are encouraging, additional studies are needed to predict with precision (i) which patients will most likely benefit from these inhibitors, (ii) the genetic and proteomic contexts in which these inhibitors will provide maximum therapeutic benefit as a single agent, or require additional sensitization *via* combination with a targeted- or chemotherapeutic agent, and (iii) exactly *which* targeted/chemotherapeutic agent will provide maximum therapeutic benefit for combination with replication stress inducers. To address these challenges in TNBC, we have attempted to gain a comprehensive understanding of how the DNA damage response pathways regulate TNBC cell survival in response to Chk1 inhibitors, by performing high throughput loss-of-function screens.

Chk1 serves an essential function during S-phase by preventing stalled replication forks from collapsing. In addition, when cells are exposed to agents that induce single strand DNA breaks or induce replication stress, Chk1 becomes activated to turn on the S- and G2-checkpoints to temporarily arrest cell cycle progression. Thus, tumor cell death due to Chk1 inhibition can occur in at least two ways. One is through replication catastrophe due to massive replication fork collapse and de-repression of late-origin firing, which results in enhanced DNA damage. The second is by mitotic catastrophe whereby cells with DNA damage bypass the S- and G2 checkpoints and move prematurely into mitosis without repairing the damage. Thus, the response of tumor cells to Chk1 inhibition as a single agent or with chemotherapy is expected to vary depending on the proficiency of DNA repair pathways and ability to sustain DNA synthesis. Chk1 inhibitors (Chk1i) have been shown to sensitize a wide variety of cancer cells to various chemotherapeutic agents, including cisplatin, irinotecan and a number of antimetabolites.

We have identified genes whose loss induce death of TNBC cells in the presence of (1) CHK1i alone, (2) chemotherapy alone or (3) CHK1i plus chemotherapy. In addition, given the role of *TP53* as the most frequently mutated gene in TNBC, we also determined whether distinct vulnerabilities could be identified in TNBC cells that are p53-proficient *versus* p53-deficient. Thus, we have also identified the top synthetic lethal interactions that are either common to both p53-proficient and p53-deficient TNBC, or unique to p53-deficient TNBC; we are currently performing *in vitro* studies to validate the identified mechanisms. We anticipate these studies to be applicable to other agents that induce replication stress and cell cycle checkpoint bypass. Ongoing *in vivo* preclinical studies, which utilize patient-derived xenografts (PDXs) of TNBC to validate these findings are expected to impact patient selection for clinical trials, and also allow us to predict which chemotherapeutic agents will be most effective for combination with different cell-cycle checkpoint inhibitors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-06-07

Title: PTEN immunohistochemistry is a predictor of mismatch repair status in breast cancer

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Body: Phosphatase and tensin homolog (*PTEN*), a potent downregulator of the PI3K-Akt pathway, has been shown to mediate the interaction between poly (ADP-ribose) polymerases (PARPs) and the mismatch repair (MMR) complex in endometrial and ovarian cancer. Drugs inhibiting PARPs (iPARPs) are currently considered promising therapeutic tools in a subset of *PTEN*-defective tumors. Regrettably, the frequency and significance of MMR alterations in breast cancer is debated, and their relationship with *PTEN* status has not been investigated in the breast. Furthermore, many of the studies on the DNA damage response and its therapeutic implications in breast cancer focus on inherited syndromes (e.g. Lynch syndrome and hereditary breast-ovarian cancer syndrome).

Aims: We sought to explore the interplay between *PTEN* and the MMR system and to define whether *PTEN* immunohistochemistry (IHC) is a predictor of MMR proficiency in non-familial breast cancers.

Methods: 373 cases of non-familial breast cancers, including a representative number of no special (n=295) and special types (n=78), carefully characterized from clinical and pathological standpoints, were reviewed and used to construct 14 tissue microarrays (TMAs). For each case, a mean of 4.5 tumor tissue cores (range 3 to 6 cores) was sampled, incorporating distinct topographic areas of the tumor, as well as matched non-neoplastic breast tissue. Taken together, 1876 spots were generated. Each TMA was subjected to IHC for *PTEN* and the DNA MMR proteins MLH1, MSH2, MSH6 and PMS2. In order to minimize human-related biases, each stained slide was digitalized and two pathologists blindly analyzed each tumor spot using a dedicated software able to segment and randomize TMA cores. The pattern of expression was therefore annotated manually on a digital database using a specific add-on module to reconstruct the original topography.

Results: According to clinicopathologic surrogate definition of intrinsic subtypes, *PTEN* protein loss or heterogeneous expression was more frequent in estrogen receptor negative cancers. Furthermore, 100% of the MMR-proficient luminal B-like (HER2+) and triple-negative breast cancers displayed strong and diffuse homogeneous *PTEN* expression, while *PTEN*-positive status identified MMR-proficient luminal A-like and luminal B (HER2-) like tumors with accuracy rates of 89.3% and 92.7%, respectively ($p=0.001$, Fisher's exact test).

Conclusions: The present study is the first to investigate *PTEN* protein loss in a large set of non-familial breast carcinomas based on their DNA MMR status by IHC. Here, we demonstrated that *PTEN* strong and homogeneous expression by IHC is able to capture the vast majority of MMR-proficient non-familial breast cancers. Our findings broaden the understanding of the biology underpinning these tumors, suggesting that *PTEN* is likely play a role in the development of MMR alterations. Given that *PTEN*-defective breast cancers have the propensity to develop additional somatic alterations in the MMR system, our results suggest that IHC for *PTEN* and MMR proteins may be employed as an ancillary study to define new subclasses of sporadic breast cancers potentially eligible for iPARPs therapies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-06-08

Title: *BRCA1* promoter hypermethylation, but not *BRCA1* expression, is associated with basal-like features and good prognosis in triple negative breast cancer

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Body: Purpose: *BRCA1* gene mutation is closely associated with familial hereditary breast cancer. Decreased expression of *BRCA1* could be detected in certain types of sporadic breast cancer without *BRCA1* mutations. Aberrant hypermethylation of DNA promoter CpG islands is one of the mechanisms by which tumor suppressor gene expression and function could be lost. We investigated *BRCA1* methylation status and *BRCA1* immunohistochemistry (IHC) expression and their clinic-pathological significance in triple negative breast cancers (TNBC).

Patients and methods: We analyzed *BRCA1* promoter hypermethylation using a methylation specific PCR assay and *BRCA1* IHC expression using the MS110 monoclonal antibody and Garg and Meisel scoring classification. Their clinicopathological and prognostic implications were analyzed in a TMA of TNBC samples from European patients.

Results: To date, 123 TNBC have been analyzed. 25 tumors (20%) presented a *BRCA1* promoter hypermethylation. *BRCA1* IHC expression was retained, equivocal and lost in 73 (59%), 17 (14%) and 33 (27%) cases respectively. No significant correlation was found between promoter hypermethylation and protein expression ($p=0.28$). A significant association was found between promoter hypermethylation and basal staining (CK5/6 and/or EGFR IHC staining), while using either a 10% or 1% cut-off for basal definition ($p= 0.04$ and 0.03 , respectively). No significant association was found between *BRCA1* expression and a basal phenotype. With a median follow-up of 6.3 years (range [0.01 – 11.8]), 35 relapses and deaths occurred (71.2% 5-years RFS and 77.8 5-years OS). RFS was significantly associated with T and N stage, and a trend was seen for a better prognosis of *BRCA1* promoter hypermethylated tumors (5-years RFS: 84% vs. 68%, $p=0.07$). OS was significantly associated with T and N stage, and adjuvant chemotherapy.

Conclusion: *BRCA1* promoter hypermethylation is associated with basal-like features and seems to be associated with a better prognosis in TNBC. *BRCA1* IHC expression is not a good surrogate marker for evaluation of the methylation status of the tumors, and did not appear associated with prognosis in our series. The data will be updated for the meeting in term of number of analyzed tumors in order to strengthen the statistical analysis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-06-09

Title: Cyclin d1 binding to chromatin and the induction of chromosomal instability requires the fuzzy domain

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Body: The *cyclin D1* gene encodes the regulatory subunit of a holoenzyme that phosphorylates and inactivates the retinoblastoma (Rb) and the nuclear respiratory factor 1 (NRF1) proteins to regulate nuclear DNA synthesis and mitochondrial biogenesis. Cyclin D1 is required for oncogene-dependent growth and genetic ablation of the murine *cyclin D1* gene resulted in resistance to Ras or ErbB2-induced mammary tumorigenesis and APC-induced gastrointestinal tumorigenesis. *Cyclin D1* overexpression occurs in human breast, prostate, lung, and gastrointestinal malignancies and its abundance is induced at the level of transcription, translation and through post-translational modifications. Cyclin D1 plays a key role in transcriptional regulation inducing gene expression governing chromosomal instability (CIN) and cell-cycle progression. Cyclin D1 is also known to bind TF regulatory regions in chromatin immuno-precipitation (ChIP) assays. Genome wide analysis of cyclin D1 occupancy using ChIP-Seq identified binding sites including both the coding and non-coding genome with enrichment for genes regulating CIN and the G₂/M phase (*Top2A*, *AurkB*, *Cenpp*, *Mlf1ip*, *Zw10*, *Ckap2*) consistent with enrichment of cyclin D1 at G₂/M and the finding that cyclin D1 induces CIN. We sought to identify the molecular mechanisms governing the recruitment of cyclin D1 in the context of local chromatin to promote CIN. In order to define the domain of cyclin D1 involved in aneuploidy and tumorigenesis, we transduced and assessed the induction of aneuploidy in MEF cells using cyclin D1 wt (wt), cyclin D1 *C-terminus* domain (C4), cyclin D1 mutant lacking of the E-box motif (ΔE) or ctrl. We also searched for potential histone protein interaction motifs in cyclin D1 and determined the epigenetic motif recognized by cyclin D1 using a histone peptide array. The recognition of an epigenetic code by cyclin D1 may facilitate genome wide expression changes during cell-cycle progression and tumorigenesis. We finally identified a “fuzzy” domain of cyclin D1 which is required to local chromatin access for regulatory promoter regions governing and promoting CIN.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-07-02

Title: ST8SIA1 regulates tumorigenesis in triple negative breast cancer

Battula VL, Nguyen K, Sun JC C, Dasgupta A, Bartholomeusz C and Andreeff M. The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Recurrence in breast cancer is mainly due to metastases and drug resistance in a fraction of primary tumors cells which are also known as cancer stem cells or tumor initiating cells. We found that Ganglioside GD2 identifies breast cancer stem cell (BCSCs) in triple negative breast cancer (TNBC) and that GD2 biosynthesis is tightly regulated by enzyme ST8SIA1 (GD3 synthase) in GD2⁺ cells. We have reported that ST8SIA1 is highly expressed in TNBC and its expression is highly correlated with p53 mutations primary tumors (Yan et al, SABCS abstract 2016). Here we hypothesize that ST8SIA1 has a functional role in BCSC mediated tumorigenesis in TNBC. To test the hypothesis, we deleted ST8SIA1 in SUM159 cells using CRISPR-Cas9 technology. As expected, deletion of ST8SIA1 in SUM159 cells reduced GD2⁺ cells from 17±1.5% to 0.3±0.1%. However, cell proliferation assay revealed no significant difference between ST8SIA1-KO and Cas9 control cells. In contrast, in-vitro tumorigenesis by soft-agar assays revealed a complete loss of colony formation in ST8SIA1-KO cells, whereas Cas9 control cells produced 30±10 colonies out of 1000 cells plated. To investigate tumor initiation potential, ST8SIA1-KO- or Cas9 control- SUM159 cells were transplanted in mammary fat pad of NSG mice. Cas9 control cells produced tumors within 1-2 weeks and reached the maximum allowed size by IACUC (1.5cm) within 3-4 weeks. In contrast, ST8SIA1-KO cells failed to produce any tumors even 15 weeks after injections. In addition, survival analysis by log-rank test revealed that most of the cas9 control cell injected mice died within 4 weeks after cell implantation whereas no deaths were observed in ST8SIA1-KO cells injected mice even 100 days after tumor implantation. These data indicate that loss of ST8SIA1 in TNBC cells depletes GD2⁺ BCSCs and inhibits in-vitro and in-vivo tumorigenesis.

To investigate gene expression changes due to loss of ST8SIA1 in CRISPR knockout cells, we analyzed mRNA expression in ST8SIA1-KO- and Cas9 control- SUM159 cells by RNAseq analysis (done in triplicates for each cell type). At p<0.05 and fold change >2, we found 1502 genes down-regulated and 842 genes up-regulated in ST8SIA1-KO- compared to cas9 control- cells. Ingenuity pathway analysis revealed that several stem cell associated signaling pathway including, wnt, stat3, NFκB, nanog and IL8 whereas tumor suppressor PTEN and p38 MAPK signaling were activated in ST8SIA1-KO- compared to cas9 control- cells. In specific, proteins associated with stem cell function including NOTCH3, PDGFRB, PDGFRA, VCAM1, CXCR4, CXCL12, SOX2, wnt5a were down regulated in ST8SIA1-KO cells whereas DKK1 which acts as an antagonist for wnt-β-catenin signaling, was up-regulated in ST8SIA1-KO cells. These findings were validated by flow cytometry and western blot analysis using specific antibodies. In conclusion, our data suggests that deletion of ST8SIA1 in TNBC cells depletes BCSCs and inhibits tumorigenesis in-vitro and in-vivo. Development of specific inhibitors of ST8SIA1 could be of potential therapeutic value for patients with TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-07-03

Title: GD2-mediated FAK signaling regulates breast cancer stem cell function in triple negative breast cancer

Nguyen K, Sun JC C, Hortobagyi GN N, Andreeff M and Battula VL. The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Ganglioside GD2 identifies breast cancer stem cells (BCSCs, Battula et al., JCI, 2012) and expression of GD2 is tightly regulated by GD3 synthase (GD3S). GD3S is highly expressed in GD2⁺ cells and inhibition of GD3S inhibits tumor formation and metastasis of breast cancer cells. However, the mechanism of GD2-mediated regulation of BCSC function is not known. Here we hypothesize that GD2 regulates signaling pathways involved in cell adhesion, migration and invasion of breast cancer cells. To identify these signaling pathways, antibody micro-arrays were used with 850 validated antibodies specific to total or phosphorylated proteins. Interestingly, focal adhesion kinase (FAK) was the most significantly phosphorylated protein in GD2⁺ compared to GD2⁻ cells (S910 and S722). In addition, expression of FAK downstream mediators including Csk, PKC α , PKC δ , Pyk2, and p38MAPK, was up-regulated in GD2⁺ compared to GD2⁻ cells. Western blot analysis of FACS sorted SUM159 cells also revealed increased phosphorylation of FAK >80% at Y397 and >25% at Y861 in GD2⁺ compared to GD2⁻ cells. FAK downstream targets including paxillin, p130 Cas, pERK were also up-regulated in GD2⁺ cells compared to GD2⁻ cells indicating definitive activation of FAK signaling in GD2⁺ BCSCs. To investigate the functional role of GD2 in FAK mediated functions, we genetically deleted GD3S using the CRISPR knock-out system in SUM159 cells. only <1% GD2 expression compared to >20% in parental cells was observed. GD3S-KO cells grew 5-10% slower in cell culture mostly because of the reduction (15 \pm 5%) in adherence. Trans-well assays revealed 3-5 fold reduction in migration and invasion of GD3S-KO compared to parental cells. These data indicate that GD2 and GD3S are not only the markers of BCSCs but also regulators of their function. Finally, we tested the effect of FAK inhibitor (PF-573228) against GD2⁺ BCSCs and GD3S-KO SUM159 cells. PF-573228 treatment decreased the number of mammospheres generated by GD2⁺ cells 3-4 fold in a dose dependent manner (100nM-1 μ M). In addition, treatment of PF-573228, inhibited migration and invasion of GD2⁺ cells 2 and 3 fold, respectively. However, treatment with PF-573228 on GD3S-KO cells further reduced their ability to migrate and invade by over 70% compared to untreated cells. In addition, GD3S-KO cells failed to form any mammospheres when cultured under low adherence conditions (p<0.00001), whereas the parental cells formed 15-20 mammospheres per 1,000 cells plated. In conclusion, our data demonstrate that FAK signaling is activated in GD2⁺ cells but that FAK inhibition alone may not be sufficient to inhibit BCSC function. Combined FAK and GD3S inhibition may exert highly synergistic effects against BCSCs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-07-04

Title: Transition from a pre-malignant lesion to cancer does not require stemness of the cancer-originating cells in the lesion

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Body: The precancerous lesion is the final step before malignancy, and is thus a key step to cancer prevention. Precancerous lesions are heterogeneous harboring distinct subsets of cells from stem cells to differentiated cells. Whether the stem cells or other subsets in these precancerous lesions are responsible for the eventual cancer remains unanswered. Here, we report that in the precancerous lesions of the MMTV-Wnt1 model of basal-like breast cancer, there exist the stem cell-enriched keratin 6a+ subset and more differentiated WAP+ cell subset. We demonstrate that both mutated Ras and B-Raf can robustly transform both cell subsets into cancer. These data suggest that multiple cell subsets in precancerous early lesions can evolve into cancer. This finding indicates that cancer prevention should target both self-renewing cells and other cell subsets in developing precancerous lesions in high-risk individuals.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-07-05

Title: BRCA1-IRIS overexpression promotes and maintains the tumor initiating phenotype in TNBC cells: Implications for breast cancer early lesions

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Body: Introduction. Tumor-initiating (TIC) phenotype is potent inducer of dissemination of cancer cells from early lesions showing self-renewal, multi-lineage differentiation, chemo-resistance, and are defined as CD44⁺/CD24⁻/ALDH⁺. Here, we demonstrate that BRCA1-IRIS overexpression (IRISOE) promotes TNBC formation by enhancing basal, EMT and TIC phenotype. In regards to TIC phenotype, we show that IRISOE elevates CD44 expression and ALDH activity while prevents CD24 surface presentation. We present a novel pathway for CD24 internalization and confirm these data in animal model and tumor samples. Finally, we evaluated in pre-clinical models the ability of IRISOE to promote dissemination from early lesion and potential utility of IRIS inhibition to deplete TICs in TNBCs.

Methods. We used western blot, qRT/PCR, immunofluorescence (IF) and IHC to establish association of IRISOE with basal, EMT and TIC phenotype. FACS analysis to determine IRISOE effect on the TIC phenotype. 2D and 3D culture to evaluate the utility of IRIS inhibition + a ligating anti-CD24 antibody on TICs. *In vivo* mice models to study early dissemination, metastasis formation and the effect of IRIS peptide on TIC tumor growth. Finally, patient cohort to correlate IRISOE to BRCA1 expression, stem and dissemination biomarkers in breast cancer patients.

Results. We demonstrate an association between IRISOE and high basal and EMT phenotype. IRISOE leads to significant increase in basal biomarkers (EGFR, CK5, CK17, CDH3) and EMT markers (N-cadherin, vimentin, fibronectin, FOXC2, twist and snail). Within a cohort of breast cancer patients (n=326), including subcohort of TNBC patients (n=72), IRISOE was associated with loss of BRCA1. Association between IRISOE and increase in TIC phenotype, *in vitro* and *in vivo* was documented. We propose EGFR-c-Src-cortactin signaling pathway induced by IRISOE to be involved in CD24 internalization and thus TIC phenotype. In support of that both western blot and IF analysis showed that while IRIS silencing in TNBC cells led to complete re-direction of cytoplasmic CD24 to the membrane, silencing of members of this pathway led to partial effect. Conversely, their overexpression also partially reversed the effect of IRIS silencing on the TIC phenotype. IRISOE could also induce tumorigenesis and metastasis in non-tumorigenic luminal A, MCF7 cells by enhancing TIC phenotype in them. Not only that, mice injected with low numbers of MCF7/IRISOE cells generate larger tumors, most likely, due to enhanced interactions with the stroma, an effect most likely not possible when large number of cells is injected instead. These tumors also showed high dissemination in the form of increased CTCs and DTCs in the blood and bone marrow, respectively. Inhibiting IRIS with specific peptide led to 80% regression of tumors developed using TICs in NSG mice and the effect was even more dramatic on the Sox2⁺-population remained within these tumors.

Conclusion. This study reveals that IRISOE cells acquire TIC, basal and EMT phenotypes along with lack of BRCA1 expression and possess high dissemination and metastasis ability. Our results support inhibiting IRIS in TNBC patients to prevent early dissemination and metastasis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-07-06

Title: A novel non-canonical Notch1-IKK α -mTORC2-AKT pathway maintains survival in triple negative breast cancer cells and cancer stem-like cells

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Body: Triple negative breast cancer (TNBC) is a heterogeneous group of clinically aggressive breast cancers. TNBC patients have high risk of recurrence and metastasis, and current treatment options remain limited. There is strong evidence for the involvement of Notch signaling in TNBC and in breast cancer stem-like cells (CSCs). Notch1 is highly expressed in Basal-like 1 (BL1) and especially Mesenchymal-Stem-Like (MSL) TNBCs. Expression of Notch1 and its ligand Jagged1 correlate with poor prognosis. Treatment of TNBC with dual mTORC1/2 inhibitors leads to resistance through activation of Notch1. Expression of Notch1 protein correlates with pAKT and nuclear NF- κ B in TNBC. Here, we demonstrate that Notch1 promotes cell survival in MDA-MB-231 cells, representative of MSL TNBC, in part by activating NF- κ B. Notch activation by Jagged1-expressing stromal cells enhances transcription of the anti-apoptotic gene cIAP-2 (BIRC3), a known NF- κ B target. This event is dependent on recruitment to the cIAP-2 promoter of NF- κ B subunits, IKK α and Notch1. Short term exposure of MDA-MB-231 cells (MSL, PTEN wild-type), but not MDA-MB-468 cells (BL1, PTEN-null) to recombinant Jagged1 leads to rapid AKT phosphorylation. This is suppressed by dual mTORC1/2 inhibitors, AKT inhibitors and IKK α inhibitors but not Everolimus (mTORC1-selective inhibitor). Rapid AKT phosphorylation downstream of Notch1 requires mTORC2, PI3K and IKK α , and contributes to NF- κ B activation. These observations support a model where canonical and non-canonical mechanisms downstream of Notch1 trigger rapid AKT phosphorylation and NF- κ B activation in PTEN wild-type TNBC cells. Both arms of this pathway require IKK α . CSCs derived from MDA-MB-231 cells have increased Notch1, pAKT and pIKK α expression. Combined pharmacological inhibition of Notch and AKT or Notch and IKK α completely blocks secondary mammosphere formation. These data and published literature suggest that: 1) IKK α connects the Notch and mTORC2/AKT pathways in some TNBC subtypes; 2) IKK α is also required for nuclear Notch1-mediated NF- κ B activation and may be a critical node in the Notch signaling network; 3) A feedback mechanism may exist in some TNBC cells between mTORC2/AKT and Notch1; 4) The non-canonical Notch-IKK α -AKT pathway has a potential therapeutic role in targeting CSCs of selected TNBC subtypes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-07-07

Title: DYRK2 contributes to the generation of breast cancer stem cells through KLF4

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Body: <Introduction>

Cancer stem cells (CSCs) have been defined by the potential to self-renew and to differentiate. CSCs pose a major hurdle in the treatment of cancer. However, the mechanisms by which cells acquire CSC properties such as drug resistance remain unclear. Dual-specificity tyrosine-regulated kinase 2 (DYRK2) is a protein kinase that phosphorylates its substrates on serine/threonine. Initially, we found that DYRK2 phosphorylates p53 at Ser 46 to regulate apoptotic cell death in response to DNA damage. Recently, we have shown that DYRK2 controls Snail degradation in breast cancer and ovarian serous adenocarcinoma. We also found that knockdown of DYRK2 in luminal-type breast cancer MCF-7 cells increased the cancer stem cell population. Kruppel-like factor 4 (KLF4) is one of the Yamanaka factors. It has been reported that pluripotent stem cells from mouse embryonic or adult fibroblasts are induced by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4. This finding led us to determine if KLF4 is indispensable for the maintenance of CSCs.

The aim of this study is to clarify whether DYRK2 regulates CSCs through KLF4 in breast cancer.

<Methods>

Cell lines: MCF-7 (human mammary carcinoma: ATCC) cells were grown according to standard protocols.

We established stable DYRK2-depleted cells. MCF-7 cells were transfected with pSuper-puro vector (pSuper control) or pSuper-puro DYRK2 shRNAs (shDYRK2) with puromycin to isolate stable cell lines.

In turn, we established both stable DYRK2- and KLF4-depleted cells. shDYRK2 cells were transfected with pSuper-neo vector (pSuper-neo control) or pSuper-neo KLF4 shRNAs (shKLF4) with puromycin and G418. Knockdown of DYRK2 or KLF4 was confirmed by real-time RT-PCR and immunoblotting.

The depleted cells were compared with the control cells using real-time RT-PCR, immunoblotting, flow cytometric analysis, mammosphere assay, xenograft models and immunohistological staining.

<Results>

We analyzed the population of breast cancer stem cells by flow cytometric analysis and *in vitro* mammosphere assay. The results showed that knockdown of DYRK2 was associated with the increase of CD44⁺/CD24⁻ cells. While pSuper control cells formed mammospheres, they did in a lesser extent compared to shDYRK2 cells.

In real-time RT-PCR and immunoblotting analysis, stable DYRK2 depletion in MCF-7 cells induced KLF4 accumulation.

We then investigated the effect of KLF4 on stemness by flow cytometric analysis and *in vitro* mammosphere assay. The results showed that knockdown of KLF4 in shDYRK2 cells reduced the proportion of CD44⁺/CD24⁻ cells. Whereas shDYRK2/shKLF4 cells formed mammospheres, they did in a lesser extent compared to shDYRK2/pSuper-neo control cells. Moreover, the scale of the mammospheres formed in shDYRK2/shKLF4 cells was significantly smaller, as compared with that in shDYRK2/pSuper-neo control cells.

In xenograft models, the loss of KLF4 protein expression significantly decreased tumor formation.

Immunohistological staining of fifty-nine samples from surgically treated breast cancer patients showed an inverse correlation between DYRK2 and KLF4 expression.

<Conclusion>

These findings revealed that DYRK2 contributes to the generation of breast cancer stem cells through KLF4.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-07-08

Title: The role of inducible nitric oxide synthase in the stemness of triple negative breast cancer

Mehibel M, Simoes BM M, Telfer B, Williams KJ J and Stratford IJ J. Experimental Oncology Group, Manchester Pharmacy School, The University of Manchester, Manchester, Greater Manchester, United Kingdom; Breast Cancer Now Research Unit, Institute of Cancer Sciences, University of Manchester, Manchester, Greater Manchester, United Kingdom and Hypoxia and Therapeutics Group, Manchester Pharmacy School, The University of Manchester, Manchester, Greater Manchester, United Kingdom.

Body: Background

Breast cancer comprises a highly heterogeneous group of malignancies, which despite advances in diagnosis and therapy, collectively remain the second leading cause of death in western women. Triple negative breast cancer accounts for 15-20% of all breast cancer cases and is associated with an overall poor prognosis. It is noteworthy that these TNBCs tend to harbour more cancer stem cells (CSCs).

Increased inducible nitric oxide synthase (NOS2) expression has been reported in breast cancer where it was found to directly correlate with the traditional prognostic markers including tumor grade, P53 status, and tumor microvascularization as well as poor breast cancer specific survival. Nitric oxide (NO) signalling has various oncogenic effects in cancer cells which include evasion of apoptosis, enhanced proliferation and chemotherapeutic resistance. There is also some evidence that nitric oxide plays a role in stem cell biology.

Our aim was therefore to evaluate whether nitric oxide confers stem cell properties to breast cancer cells and whether pharmacological inhibition of NO production in these tumors could be a promising strategy in successful targeting of breast cancer stem cells.

Method

To achieve this goal, basal-like human breast cancer cell lines with different levels of nitric oxide were studied by sorting breast cancer cells using flow cytometry. Three methods for identifying putative cancer stem cells were used: *i*) qRT-PCR to investigate the expression of cancer stem cell markers (ALDH1, Sox2, Oct4, Nanog) *ii*) culture of mammary cells in anchorage independent conditions (mammosphere assay) and *iii*) co-expression of NOS2 and stem cell markers in tumor xenografts. In parallel, cells were engineered to constitutively overexpress the NOS2 gene and the effect of pharmacological inhibition of nitric oxide release on stemness characteristics of these cells was assessed.

Results

Basal-like breast cancer cell lines, producing high levels of nitric oxide, show enhanced stem cell properties as defined by mRNA expression level of the cancer stem cell markers ALDH1, Nanog and Oct4. Within each basal-like cell line studied, the NO^{high} subpopulation showed higher levels of mammosphere forming efficiency (MFE) compared with the NO^{low} cells. Furthermore, pharmacological inhibition of nitric oxide in the NOS2 overexpressing cells led a significant reduction in the mammosphere forming efficiency of the cells and in MDA231 tumor xenografts, there was an obvious co-localization between NOS2 expression and the stem cell marker ALDH1A1.

Conclusion

Our study suggests that nitric oxide can be a promising determinant of breast cancer stem cell activity in basal-like cell lines and we propose that drugs modulating expression and/or activity of NOS2 may be used to reduce heterogeneity in tumors and could be of therapeutic potential.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-07-09

Title: Stem cell-like transcriptional reprogramming in metastatic resistance

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Body: Whether an aggressive phenotype is promoted through adaptation or resistance to target inhibition remains poorly understood. Here, complementary studies in human tumors, xenograft models and cell lines reveal transcriptional reprogramming that supports metastasis in response to PI3K/mTOR inhibition. This feature is driven by stem cell-like transcription factors. These factors functionally cooperate to promote transcriptional up-regulation of key mTOR pathway components and of metastasis mediators. Their expression is also associated with stem cell-like and metastasis signatures in tumors, and their depletion impairs the metastatic potential of breast cancer cells.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-07-10

Title: MiR-146a functions as suppressive non-coding gene via indirect upregulation of Let-7 to promote asymmetric division and inhibit the self-renewal ability of breast cancer stem-like cells

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Body: MiR-146a plays as diverse roles in systemic malignancies, stimulating the tumor growth or blocking the tumor proliferation. However, its roles in breast cancer stem-like cells are barely known. We first identified the suppressive role of miR-146a in stem cells' renewal, promoting the asymmetric division of stem-like cells, and the expression of miR-146a was positively related with Let-7b level in vitro and in clinical specimens. Previous studies of Let-7 revealed its suppressive functions in stem-like cells expansion, and miR-146 was predicated to target and bind to the 3'UTR of LIN28, a negative regulator of Let-7 maturation. By using luc-assay and western, results showed that miR-146a increased the Let-7b level through degrading Lin28, and Lin28 is required for miR-146a induction of stem cells arresting and more asymmetric stem cells division. Moreover, Let-7 controlled Wnt signaling pathway activity is governed and strengthened by miR-146a, contributing to decrease the ratio of stem-like cells, forcing stem cells dividing asymmetrically. MiR-146a in turn formed negative feedback loop with Let-7 via the repression of NF- κ B and Snai1 caused by Let-7b. Our results suggested the possible miR-146a/LIN28/Let-7/Snai1 signaling pathway in restraining the symmetric cells division, which was referred to the self-renewal capacity of breast cancer-stem like cells, and this axis helps to prohibit long-term tumor resistance and recurrence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-07-11

Title: The ectopic FOXA1 expression correlates to the luminal breast cancer stem cells

Tachi K, Shiraishi A, Bando H, Yamashita T, Tsuboi I, Kato T, Ichioka E, Tsushima Y, Ikeda T, Iguchi A, Hara H and Ohneda O.
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Body: Background: It is known that ER-positive breast cancer constitutes approximately 70% of all breast cancer and the particular population of these, called as luminal A breast cancer can lead to late recurrence, possibly due to the presence of dormant tumor cells. It is also argued that there are many similar biological characteristics between dormant cells and cancer stem cells (CSCs). So we hypothesized that some part of CSCs would be involved in the late recurrence of luminal type breast cancer and looked for the factors correlating late recurrence by comparing CSC-enriched population and other population.

Materials and Methods: We used the MCF-7 and HCC1500 human breast cell lines. Moreover, we isolated the BC#1 breast cancer cell from metastatic pleural effusion of a breast cancer patient (79 years old, ER+, PgR+, HER2 score 0). These cells are cultured in the adhesion culture condition or the mammosphere culture condition. The immunohistochemical characteristics such as ER, PgR, HER2, Cytokeratin (CK) 8, CK5/6 status of these cells were confirmed. The analysis of the ALDH activity and the CD44+/24- expression as the CSC marker were carried out using flow cytometry. The expression of target genes was analyzed using quantitative RT-PCR. Protein expression was determined by western-blotting. To assess the self-renewal potential of CSCs and the proliferation of immature cancer cells, we used mammosphere formation assay and colony formation assay. To investigate the effects of 4-hydroxytamoxifen(4-OHT) on mammosphere formation assay, 1 μ M of 4-OHT added to the medium in the particular experiment. **Results:** BC#1 was confirmed as luminal type by immunohistochemistry and qRT-PCR. The expression tendency between two CSC markers are almost same and correlated to the mammosphere formation capacity. We also confirmed that mammosphere culture have an increased ALDH-positive population compared with adherent culture cells. Using flow cytometry, we divided the cell lines and isolated cells into two populations, ALDH-positive and negative. By qRT-PCR analysis, ALDH-positive population showed higher expression of FOXA1 (ER expression related gene) than ALDH-negative population. In addition, we found the significantly higher expression of *FOXA1* gene, and *RPRM* gene (p53 induced G2/M arrest related gene) in mammosphere culture samples than in adhesion culture samples. In terms of the effects of 4-OHT, 4-OHT resistant mammosphere samples showed significantly increased FOXA1 gene expression level. Finally, we established shFOXA1 MCF-7 cells to investigate the relationship between self-renewal potential and FOXA1 expression. We found that colony formation in shFOXA1 MCF-7 cells decreased compared with control while there is no significant difference in the number of mammospheres. **Conclusion:** CSC-enriched population showed higher FOXA1 expression and our results suggested that the expression of FOXA1 correlate to the proliferation of immature breast cancer cell rather than the induction of self-renewal potency of CSCs. Moreover, there is no report argued about the correlation between CSCs and G2/M arrest related gene like *RPRM* so that the biological mechanism should be investigated in future.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-07-12

Title: RAD51AP1 is a novel prognostic marker and therapeutic target for breast cancer

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Body: Background: Ionizing radiation is one of the most effective therapeutic strategies for the treatment of breast cancer and is considered as a more appropriate therapy for patients with high-risk of recurrence. Despite substantial benefits are achievable with this treatment, especially for ductal carcinoma and early invasive cancer, the critical barrier in using this treatment strategy is that tumor cells develop radioresistance, which in turn progress into advanced invasive cancer. Breast cancer stem cells (BCSCs), a subpopulation of cells within the tumor with a characteristic feature of self-renewal, play a critical role radioresistance and treatment failure. BCSCs exhibit increased DNA repair activity by increasing RAD51AP1 for their prolonged survival and to evade from the radiation therapy. We explored the expression profile of RAD51AP1 in BCSCs, human normal and various subtypes of breast tumor tissues and cell lines and response to chemo- and radiation- therapy.

Methods: Gene expression (RNA and protein) profile was assessed using semi-quantitative and real-time PCR (qPCR) and western blot analyses. RAD51AP1 expression and its prognostic value in large cohort of human samples were analyzed by TCGA, GOBO, and Kaplan-Meier plotter integrative bioinformatics interface analyses. Breast cancer stem cell (BCSC) status was analyzed by FACS using CD24 and CD49f cell surface marker. Cell death was analyzed by propidium iodide (PI) stained cell cycle analysis.

Results: We found that tumor propagating CD49f⁺CD24⁺ cells activate RAD51AP1 more promptly than non-tumorigenic CD49f⁺CD24⁻ cells and confer chemo- and radiation- therapy resistance. RAD51AP1 inactivation facilitates chemo- and radiation-therapy response by depleting CD49f⁺CD24⁺ cells with significant activation of apoptotic cell death signaling. RAD51AP1 expression was significantly higher in BC, especially in the basal triple-negative and HER2-positive BC subtype, than in normal mammary tissue. Further, RAD51AP1 expression is highest in **grade III** histological tumor types and negatively associated to overall disease-free survival. RAD51AP1 levels across different BC cell lines showed that triple-negative breast cancer (TNBC) cell lines expressed highest level of this gene than other sub types.

Conclusion: Overall, our findings provide evidence that BCSCs utilize DNA repair signaling for their self-renewal and RAD51AP1 play a critical role in BCSC self-renewal and maintenance. Further, RAD51AP1 expression profile can be used as a prognostic marker to monitor disease progression and chemotherapy response.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-07-13

Title: Identification of a cancer stem cell-specific function for the histone deacetylases, HDAC1 and HDAC7, in breast and ovarian cancer

Ince TA A, Witt AE E, Lee C-W, Lee TI I, Azzam DJ J, Wang B, Caslini C, Petrocca F, Grosso J, Jones M, Cohick EA A, Gropper AB B, Wahlestedt C, Richardson AL L, Shiekhattar R and Young RA A. Interdisciplinary Stem Cell Institute, Braman Family Breast Cancer Institute, and Sylvester Comprehensive Cancer Center, Miller School of Medicine, University of Miami, Miami, FL; Brigham and Women's Hospital, and Harvard Medical School, Boston, MA; The Whitehead Institute for Biomedical Research, Cambridge, MA; University of Miami Miller School of Medicine, Miami, FL; Boston Children's Hospital, and Harvard Medical School, Boston, MA and University of Miami Miller School of Medicine, Sylvester Comprehensive Cancer Center, Miami, FL.

Body: Tumors are comprised of a highly heterogeneous population of cells, of which only a small subset of stem-like cells possess the ability to regenerate tumors *in vivo*. These cancer-stem-cells (CSCs) represent a significant clinical challenge as they are resistant to conventional cancer therapies and play essential roles in metastasis and tumor relapse. Despite this realization and great interest in CSCs, it has been difficult to develop CSC-targeted treatments due to our limited understanding of CSC biology.

Multiple signaling pathways involved in the regulation of CSCs have been identified. However, the regulation of CSCs is unlike the reversible short-term changes in cellular phenotype induced by various extracellular factors, or the permanent changes induced by mutations. The hierarchical differentiation of CSCs to nsTCs is long-lasting over many cell generations but it is also reversible, that is more akin to tissue differentiation, which suggests that epigenomic factors such as histone modifications may also be involved in the regulation of the CSC phenotype

Here, we present evidence that specific histone deacetylases (HDACs) play essential roles in the breast CSC phenotype. HDACs are chromatin-modifying enzymes that are involved in regulation of differentiation, autophagy, apoptosis, migration, mitosis, and angiogenesis. There are 11 different HDAC genes with tissue-specific expression. Utilizing a novel breast CSC model, composed of isogenic BPLER (CSC) and HMLER (nsTC) cell line pairs, we discovered that among the 11 family members, only HDAC1 and HDAC7, are specifically over-expressed in breast CSCs when compared to non-stem-tumor-cells (nsTCs). Furthermore, we determine that HDAC1 and HDAC7 are necessary to maintain breast CSCs, and that over-expression of HDAC7 is sufficient to augment the CSC phenotype. Interestingly, while HDAC1 and HDAC7 have been implicated in the regulation of normal pluripotency and ES cells, their role in the regulation of malignancy in particular with breast and ovarian CSCs have not been appreciated before.

We also demonstrate that HDAC inhibitors (HDACi) targeting HDAC1 and HDAC7 such as MS275 and MGCD0103 can be used to preferentially target breast and ovarian CSCs. So far, the limited clinical studies have been completed with these HDACi, predominantly in Leukemia and Lymphomas. Our results indicate that these drugs should be tested more extensively in solid tumors. Furthermore, in standard trial design with cytostatic and cytotoxic drugs, the reduction in tumor size has been one of the main metrics for monitoring patient response. However, CSC targeting drugs may not cause a rapid reduction in tumor size. Since the CSC-specific actions of these HDACi (MS275 and MGCD0103) were previously unknown, CSC biomarkers were not used in patient selection or for measuring patient response. Our results suggest that biomarkers such as HDAC1 and HDAC7 are particularly compelling as a supplement to tumor size measurements for patient selection and monitoring therapy response. Since we found that the regulation of HDAC7 is predominantly occurring at the protein level, it would be feasible to use HDAC7 immunohistochemistry to stratify patients in future clinical trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-01

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-02

Title: Improving the AJCC breast cancer staging system by incorporating tumor biomarkers

Chavez-MacGregor M, Mittendorf EA A, Clarke CA A, Lichensztajn DY Y, Hunt KK K and Giordano SH H. MD Anderson Cancer Center and Cancer Prevention Institute of California.

Body: BACKGROUND: The current American Joint Committee on Cancer (AJCC) breast cancer staging system provides important prognostic information, however its use is limited by the lack of data incorporating prognostic and predictive biological markers. In this study we sought to determine the relationship between stage, breast cancer subtype, grade and outcome in a large population-based cohort, and to develop a risk score point-based system incorporating biological factors to the current AJCC staging system.

METHODS: Patients diagnosed with primary breast cancer stage I-IV, between 2005-2008 were identified in the California Cancer Registry. For patients with stage I-III disease, pathological stage was recorded. For those with stage IV, clinical stage was used. 5 year-breast cancer specific survival (BCSS) and overall survival (OS) rates were determined for each potential TNM combination according to breast cancer subtype. Cox proportional hazard models were used to identify independent predictors of outcome. A risk score point-based system (range 0-3 points) was created to complement the current anatomic AJCC staging system. One point was assigned for each one of the following tumor characteristics: hormone receptor (HR)-negative status, HER2-negative status and grade 3. Survival probabilities between groups were compared using log-rank test. Multivariable analysis models according to stage and risk score were performed for BCSS and OS.

RESULTS: A total of 43,938 patients were included. The 5-year BCSS and OS for each TNM combination differed according to breast cancer subtype. The best outcomes were seen among HR-positive patients followed closely by those with HER2-positive and HR-positive tumors with the worst outcomes observed among patients with triple negative tumors. In a multivariable model, after adjusting for stage, treatment variables and other important confounders, ER negative status (OR 2.14; 95%CI 1.98-2.30), HER2-negative status (OR= 1.24; 95%CI1.14-1.34) and grade 3 (OR=2.03; 95% CI 1.88-2.20) were independent predictors of BCSS. Our risk score system separated patients into 4 risk groups within each stage category (all P<0.05). Similar results were seen for OS. The results in the table show that combining stage and risk score provides improved prognostic information.

Hazard ratios for BCSS according to stage and risk score.

Stage/Risk Score	Hazard Ratio	95%CI
I-0	Reference	
I-1	0.63	0.42-0.97
I-2	2.81	1.87-4.23
I-3	4.90	2.79-8.61
IIA-0	3.65	2.26-5.91
IIA-1	2.24	1.50-3.33
IIA-2	5.87	3.94-8.73
IIA-3	9.35	6.24-13.99
IIB-0	4.76	3.13-7.24
IIB-1	5.04	3.37-7.53
IIB-2	9.46	5.63-15.92
IIB-3	14.59	9.78-21.79
IIIA-0	9.13	6.12-13.62
IIIA-1	7.38	4.92-11.06
IIIA-2	12.01	7.14-20.19
IIIA-3	32.54	21.59-49.04

IIIB-0	18.97	12.62-28.52
IIIB-1	19.05	12.68-28.61
IIIB-2	28.34	18.91-42.47
IIIB-3	35.61	21.33-59.43
IIIC-0	11.52	5.84-22.74
IIIC-1	15.76	6.07-40.94
IIIC-2	26.77	16.74-42.83
IIIC-3	56.63	36.60-87.60
IV-0	49.93	30.64-81.36
IV-1	67.45	45.36-100.23
IV-2	107.62	72.36-60.07
IV-3	164.04	107.18-251.04

CONCLUSIONS: Incorporating biological factors to the current AJCC staging system provides more accurate prognostic information and reflects current treatment practice. The proposed risk score improves the AJCC breast cancer staging system and should be incorporated in the upcoming revision.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-03

Title: Predictors of aggressive end-of-life care in women metastatic breast cancer

Accordino MK K, Wright JD D, Vasan S, Neugut AI I, Hillyer GC C and Hershman DL L. Columbia University College of Physicians and Surgeons, New York, NY and Columbia University Mailman School of Public Health.

Body: Background: Despite recommendations against aggressive end-of-life (EOL) care, a high percentage of patients with metastatic breast cancer (MBC) receive aggressive EOL care. MBC is a heterogeneous disease with a wide variation in survival. EOL care may differ by the patients' long-term course of care. We performed a population-based analysis to evaluate patterns and predictors of aggressive EOL care and associated costs among women with MBC.

Methods: The Surveillance, Epidemiology, and End Results-Medicare database was used to identify female patients with MBC diagnosed between 2002 and 2011. Aggressive EOL care in the last month of life was identified using claims data. Specifically: ≥ 2 emergency department (ED) visits, ≥ 2 hospital admissions, >14 days hospitalized, admission to the intensive care unit (ICU), admission to hospice within 3 days or less before death, and receipt of intravenous (IV) chemotherapy in the last 14 days of life were evaluated. Direct healthcare costs in the last month of life were calculated from Medicare claims. Patients were categorized into prognosis quartiles based on survival time from diagnosis. Multivariable analysis was performed to identify patient characteristics associated with aggressive EOL care and characteristics associated with high direct healthcare expenditures in last month of life in women with hormone-receptor (HR)+ and HR- MBC. High expenditures were defined as median costs $>75^{\text{th}}$ percentile. Factors associated with high expenditures were evaluated using linear regression.

Results: We identified 5,064 eligible patients. Of these, 2,156 (42.6%) received at least one measure of aggressive EOL care in the last month of life. The most frequent aggressive EOL care received in the last month of life were ICU admissions (17.3%) and >1 ED visits (14.1%). Median cost of care in the last month of life was \$7,973. Predictors of aggressive EOL care included year of diagnosis (OR 1.04, 95% CI 1.02-1.06), black race as compared to whites (OR 1.50, 95% CI 1.25-1.79), being married compared to single (OR 1.15, 95% CI 1.01-1.32), and a Charlson comorbidity score of ≥ 2 compared to no comorbidities (OR 1.52, 95% CI 1.32-1.75). Predictors of not receiving aggressive EOL care included age >74 compared to ages 70-74, receiving care in the Midwest compared to the East (OR 0.82, 95% CI 0.70-0.96), and best prognosis compared to worst prognosis (OR 0.46, 95% CI 0.39-0.55). Predictors of high last month of life expenditures were similar in both the HR+ and HR- subsets; receipt of more aggressive EOL care was also associated with higher expenditures in both HR subsets (OR 5.02, 95% CI 3.88-6.49; OR 5.43, 95% CI 3.41-8.65, respectively). Median last month of life expenditures were unchanged from 2002-2012 for the whole population (\$7,658 to \$5,910, $p=0.93$), but rose significantly in patients in the worst prognosis quartile (\$9,236 to \$16,926, $p<0.0001$)

Conclusion: Patients with MBC frequently received aggressive EOL care. Women with poor prognosis were more likely to receive aggressive EOL care and have higher expenditures in the last month of life. Given the rising costs of cancer care, efforts should be made to identify patients early for EOL interventions to reduce costs, particularly in women with a poor prognosis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-04

Title: Overview of breast cancer mortality trends in the world

Pizot C, Boniol M, Boyle P and Autier P. University of Strathclyde Institute of Global Public Health, Lyon, France and International Prevention Research Institute, Lyon, France.

Body: Background: Since the 1990s, important changes in the detection and management of breast cancer have taken place. We analysed breast cancer mortality trends from 1989 to 2012 in 47 countries with data available for most years since 1987.

Methods: Breast cancer deaths and populations were extracted from the WHO mortality database. Age-standardised mortality rates were computed using the World standard population over the period 1987-2012 for women of all ages and for women aged <50 years, 50-69 years and ≥ 70 years. Percent changes in mortality trends were assessed over the period 1989-2012. Mortality rates are reported per 100,000 women. We constituted groups of comparable countries that are located in same region, have similar economic status and same mortality rates in 1987-89.

Results: Annual breast cancer mortality rates in 1987-89 ranged from 2.6 in South Korea to 29.3 in England and Wales (median rate of 18.5). In 2010-12, mortality rates ranged from 5.1 in South Korea to 18.4 in Denmark (median rate of 14.8). From 1989 to 2012 (23 years), declines in breast cancer mortality were observed in 39 out of 47 countries. Mortality changes ranged from a -45% reduction in England and Wales to a 79% increase in South Korea (median change of -28%). Mortality declines were more pronounced in countries with high mortality in 1987-89. In groups of comparable countries, sharp contrasts in mortality changes were observed, for instance -21% reduction in France against -37% reduction in Spain, or 5% increase in Latvia against -17% reduction in Slovakia. Although the mortality rates in 1987-89 were 20.9 in Australia and 27.4 in New Zealand, a mortality reduction of -38% was observed in both countries. Of note, in these groups of comparable countries, reductions in mortality were the same in countries that introduced mass breast screening around 1990 than in countries where breast screening was introduced in 2005 or after. Regarding age groups, the largest declines in mortality were observed in women less than 50 in all the countries except New Zealand and Hong Kong, and only three countries had an increased mortality in this age group (Brazil, Colombia, South Korea). Mortality changes in young women ranged from -59% reduction in Slovenia to 32% increase in South Korea (median change of -45%). Mortality changes in young women were not different in countries where breast screening before age 50 has always been uncommon (e.g., Norway, England and Wales) or is widespread since the late 1980's (e.g., the USA, Sweden). In women aged 50-69 years, mortality changes ranged from -49% reduction in England and Wales to 111% increase in South Korea (median change of -27%). In women aged 70 years or more, mortality changes ranged from a -33% reduction in the Netherlands to a 151% increase in South Korea (median of -11%).

Conclusions: Huge disparities in changes in breast cancer mortality rates are observed around the World, and across age groups. Downward trends in breast cancer mortality prevail in most of Europe, North America, Oceania, and in few countries of Latin America and Asia. The situation in high income Asian countries is not easy to interpret because access to efficient therapies is commonplace. There seems to be no discernible influence of screening on mortality trends.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-05

Title: Prospective family cohort analyses of gene-environment interactions in breast cancer: Body mass index

Terry MB, Dite GS S, Phillips K-A, Andrulis IL L, John EM M, Daly MB B, Buys SS S and Hopper JL L. Columbia University Mailman School of Public Health, New York, NY; The University of Melbourne, Carlton, Victoria, Australia; Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia; Mount Sinai Hospital, Toronto, ON, Canada; Cancer Prevention Institute of California, Fremont, CA; Stanford University School of Medicine, Stanford, CA; Fox Chase Cancer Center, Philadelphia, PA and University of Utah School of Medicine, Salt Lake City, UT.

Body: It is becoming possible to better identify women at increased genetic risk of breast cancer, but it is not known whether associations with risk factors are the same for women with different genetic susceptibilities. If there is a difference in disease associations by genetic risk, prevention and screening measures can be better targeted to appropriate groups of women. If there is no difference and the study is well-powered, advice to women regarding specific risk factors can be confidently given to women across the spectrum of genetic risk.

We examined this issue of gene-environment interaction for body mass index (BMI), a potentially modifiable risk factor, and used multi-generational family cancer history to derive a proxy measure of a woman's underlying genetic risk. This issue is complicated because the BMI risk association depends on time of life and potentially by menopausal status. Higher BMI has been found to be associated with an increased risk of breast cancer for post-menopausal women, especially for those 15 years or more post-menopause or aged 60 years or older. In contrast, for pre-menopausal women, young women and even adolescent girls, greater BMI has been found to be associated with a decreased risk of breast cancer.

For these analyses, we studied 11,700 participants from 5,400 families enrolled in the Breast Cancer Family Registry (North America and Australia) and the Kathleen Cuninghame Foundation Consortium for Research into Familial Breast Cancer (Australia). These prospective cohorts, enriched for familial risk of breast cancer, were established in the 1990s and now have had up to 20 years follow up (mean follow-up 10.5 years; standard deviation 4.4 years). There were 540 incident breast cancers with a mean diagnosis age of 57.2 years (standard deviation 12.1 years).

We used Cox proportional hazards models to investigate whether the associations between risk of breast cancer and BMI, measured by hazard ratios (HRs), differed by underlying genetic risk, defined as the 5-year risk of breast cancer estimated by the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) adjusted for age and continent. First, we found that the strength and direction of the association of BMI with risk depended on age at baseline ($p=0.004$). This strong interaction was not changed after adjusting for menopausal status ($p=0.5$). The change from decreased risk to increased risk was gradual over adult life with no apparent perturbation attributed to menopausal status.

Second, we found that there was a strong association with genetic risk ($p<0.001$), and this was unchanged after adjusting for the age-dependent association with BMI.

Third, we found no evidence for an interaction between BMI as a function of age at baseline and genetic risk ($p=0.8$).

Our finding of a lack of an interaction with genetic risk is important because it supports, at least for BMI, current practice that assumes that breast cancer risk factors relevant to women in the general population also apply to women of higher genetic risk. In terms of potentially reducing absolute risk, increased BMI could be more important for women at higher genetic risk. This demonstrates the power of a cohort enriched for women at increased genetic risk.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-06

Title: Impact of a decision-support tool on the utilization of colony-stimulating factors and incidence of febrile neutropenia among patients with breast cancer

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Body: Background:

Following the 2012 American Society of Clinical Oncology's *Choosing Wisely* campaign recommendation against the routine use of colony-stimulating factors (CSFs) for the primary prevention of febrile neutropenia (FN) in patients with $\leq 20\%$ risk, an evidence-based clinical decision-support tool (CSF decision tool) was implemented to promote risk-appropriate CSF use in breast cancer patients receiving chemotherapy by a national payer in the United States (US). We hypothesized that there should be no change in pre- and post-implementation FN rates if the CSF decision tool had promoted appropriate FN risk stratification among breast cancer patients.

Methods:

A retrospective observational cohort study design was used to analyze data from a national payer administrative claims database of nearly 40 million lives geographically spread across the US. The CSF decision tool was first implemented in 2014, with a staggered implementation across states (July 1, 2014 - November 1, 2014). Study subjects were female patients, aged ≥ 18 years, who initiated chemotherapy for breast cancer in the time periods before or after the implementation of the decision tool (July 1, 2014 through March 30, 2015). Patients were assigned to case (defined as patients in the states where the CSF decision tool had been implemented) or control (defined as patients in states where the CSF decision tool had yet to be implemented) cohort. Patients in each cohort were followed up to 6 months after the first chemotherapy dose in the pre- and post-implementation periods. The outcomes were changes in the incidence of FN and CSF use rates, respectively. Rates of FN and CSF use were compared between the cohorts using difference-in-differences models; generalized estimating equations were used to adjust for differences in baseline risk factors including age, history of neutropenia or infections.

Results:

The final study population comprised 7,224 patients: 4,001 and 3,223 in the case and control cohorts, respectively. There was a higher proportion of patients who were 65 years or older in the case cohort compared to the control (22% vs 18%, $p < 0.001$). Otherwise, the cohorts were comparable in FN risk factors at baseline in pre- and post-implementation periods. In adjusted regression results, pre- and post-implementation FN rates were not significantly different for both case (5.38% to 5.65%) and control (5.07% to 5.13%) cohorts, [$p=0.778$]. Use of CSF in the pre- and post-implementation periods decreased from 75% to 69% in the case cohort compared with a reduction from 72% to 71% in the control cohort: an absolute difference of 5.4% decrease in CSF use associated with the implementation of the decision support tool [$p= 0.006$].

Conclusion:

Despite a modest reduction in CSF use, we found no evidence of an increase in FN rates after the implementation of the CSF decision tool. Given the lack of impact of the *Choosing Wisely* campaign on inappropriate CSF use; our findings suggest that beyond the educational efforts and media campaigns, a greater reduction in unnecessary CSF use can be achieved through the use of clinical decision algorithms to reduce practice variation and improve adherence to national guideline recommendations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-07

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-08

Title: Baseline (BL) characteristics, treatment (tx) patterns, and outcomes in patients with hormone receptor (HR)+ vs HR– HER2+ disease from the SystHERs registry

Cobleigh M, Yardley DA A, Brufsky A, Rugo H, Swain S, Kaufman PA A, Tripathy D, Mayer M, Hurvitz S, O'Shaughnessy J, Mason G, Chu L, Antao V, Beattie M, Yoo B and Jahanzeb M. Rush University Medical Center; Sarah Cannon Research Institute and Tennessee Oncology, PLLC; University of Pittsburgh Cancer Institute; University of California San Francisco Helen Diller Family Comprehensive Cancer Center; Washington Cancer Institute, MedStar Washington Hospital Center; Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center; University of Texas MD Anderson Cancer Center; AdvancedBC.org; UCLA Jonsson Comprehensive Cancer Center and Translational Research in Oncology; Baylor Charles A. Sammons Cancer Center, Texas Oncology, US Oncology; Inflammatory Breast Cancer Research Foundation; Genentech, Inc. and University of Miami Sylvester Comprehensive Cancer Center.

Body: Introduction HR+ metastatic breast cancer (MBC) generally has a more indolent course than HR– MBC. Less is known about differences in BL characteristics and disease outcomes by HR status in HER2+ MBC in the modern tx setting where patients (pts) may recur after adjuvant therapy. SystHERs is an ongoing, prospective, observational cohort study of HER2+ MBC pts. Here we describe BL characteristics, tx patterns, and outcomes by HR status and MBC diagnosis type.

Methods SystHERs enrolled pts aged ≥18 years and within 6 months of MBC diagnosis. Locally-determined HR status was captured at initial diagnosis and/or disease recurrence; HR+ was defined as ER+ and/or PR+ disease in primary (early BC) or MBC. Tx data are shown for pts ≥9 months from MBC diagnosis to capture the completion of chemotherapy and the addition of hormonal therapy during maintenance tx. Median overall survival (OS; Kaplan-Meier) and the hazard ratio (Cox regression) were estimated.

Results As of Feb 2016, data were available for 872 eligible pts with known HR status; 70% had HR+ disease and 50% had de novo MBC. Median follow-up from MBC diagnosis was 16.5 months (range, 0.4–49.4 months). Clinically important BL demographics and disease characteristics with noticeable differences are presented in Table 1. More black (vs white) pts were observed to be HR–. More recurrent (vs de novo) pts were observed to be HR+.

MBC tx choices may be influenced by adjuvant tx. Table 2 shows the most common first-line tx regimens in de novo vs recurrent pts with HR+ HER2+ disease and ≥9 months from MBC diagnosis. Among these pts, 60% de novo and 56% recurrent pts received any hormonal therapy. The most common first-line tx regimen for pts with HR– disease was chemotherapy + HER2-targeted therapy (92%).

There were 88 (15%) and 55 (21%) deaths in the HR+ and HR– groups, respectively. Median OS was not reached for either group, but the hazard ratio favored pts with HR+ disease (log-rank $P=0.026$; hazard ratio 0.683; 95% CI 0.488–0.957).

Table 1. BL characteristics

	HR+ HER2+ (n=608)	HR– HER2+ (n=264)
Race, %		
White	82	72
Black	13	21
MBC diagnosis type, %		
De novo	47	58
Recurrent*	53	42
Visceral disease, %	63	75

No significant differences in other characteristics by HR status (eg, sex, ethnicity, education, menopausal status, performance status, number of metastatic sites at diagnosis). *Pts with prior adjuvant therapy: HR+, 89%; HR–, 83%.

Table 2. Most common first-line tx regimens in pts with HR+ HER2+ disease and ≥ 9 months from MBC diagnosis

	De novo (n=227)	Recurrent (n=235)
Chemotherapy + HER2-targeted therapy + hormonal therapy, %	49	38
Chemotherapy + HER2-targeted therapy only, %	37	41
HER2-targeted + hormonal therapy only, %	10	14

Conclusions In SystHERs, 70% of pts with HER2+ MBC had HR+ disease. A higher percentage of recurrent pts had HR+ disease vs de novo pts, perhaps suggesting selective elimination of HR- clones during adjuvant tx. While black pts are known to be more likely to develop HR- HER2- BC, our results show that HR- HER2+ disease is also more common in black pts, suggesting a proclivity for this population to develop HR- MBC regardless of HER2 status. In these early results, survival was higher in pts with HR+ HER2+ MBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-09

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-10

Title: Stage-at-presentation and time-to-treatment for American Indian patients diagnosed with breast cancer

Algan O, Campbell JE E and Algan SM M. College of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK; Biostatistics and Epidemiology, College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, OK and College of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Body: Purpose: To review the stage of presentation and time-to-treatment for American Indian (AI) patients diagnosed with breast cancer (BC) and compare them to White (W), Black (B), Asian (AS) and Hawaiian/Polynesian (HP) population across the US and by geographic region using a large national database.

Methods: The NCDB, a joint project of the American Cancer Society and the Commission on Cancer, is a comprehensive national database that registers approximately 70% of cancer patients in the US. Data for patients with BC was extracted from the NCDB 2013 PUF file (2004-2013). Categorical data were compared using the chi-square test. Time-to-treatment (TtT) analysis was performed in patients with non-metastatic breast cancer. All interval parameters were evaluated as continuous variables and compared using ANOVA analysis.

Results: Table 1 shows the number of patients, median age and stage at presentation by race. The median age was lowest for AS and largest for W patients. Compared to W patients, AI patients were less likely to present with stage 0/I disease and more likely to present with stage II/III disease. Similarly, AI patients were 25% more likely to present with metastatic disease when compared to W patients. Table 1 also shows the mean intervals (in days) from diagnosis to treatment. This analysis revealed significant differences amongst the races, with AI patients tending to have the second shortest TtT. Evaluation of the interval to treatment amongst the 9 geographic regions also revealed significant differences. The West North Central and East South Central regions had the shortest TtT and the Pacific and North East regions had the longest. For AI patients, the shortest TtT occurred in the West North Central and South Atlantic while the longest TtT occurred in the North East and West South Central regions. In general, the magnitude of these differences was under 20%, but varied greatly depending on the variables and regions evaluated. These differences were statistically significant (p-value < 0.01).

Conclusion: AI patients presented with later stage disease and longer interval to treatment for all modalities compared to White race, but did better overall than Black patients, both for stage at diagnosis and time to treat. There was considerable regional variation in time intervals across all races. Efforts to improve outcomes should be focused on addressing regional variations in time to treat, and in earlier diagnosis of breast cancer for at risk populations.

Table 1

Clinical Parameters	American Indian	White	Black	Asian	Hawaiin / Polynesian	p-value
N	4,634	1,623,175	217,696	55,942	3,473	
Age (Median)	57	61	58	55	56	< 0.001
Stage 0 (%)	17.4	20.5	21.4	25.5	19.5	< 0.001
I	35.9	42.0	31.1	37.3	35.8	
II	31.5	25.1	29.0	25.8	29.5	
III	10.7	8.7	12.4	8.5	10.8	
IV	4.6	3.7	6.1	2.9	4.3	
Interval from Diagnosis to: [Mean(Std Dev) in days]						
Treatment Start	32.3 (38.8)	27.8 (39.3)	35.0 (39.4)	31.1 (31.9)	31.2 (32.9)	< 0.001
Definitive Surgical Procedure	52.7 (62.8)	44.5 (52.5)	61.6 (71.1)	51.4 (57.3)	52.0 (60.6)	< 0.001
Start of Radiotherapy	152.9 (90.5)	128.0 (83.7)	165.4 (99.7)	144.2 (90.0)	151.0 (93.2)	< 0.001
Start of Chemotherapy	76.0 (53.1)	68.8 (47.8)	77.7 (56.1)	72.9 (52.6)	78.8 (50.2)	< 0.001

Start of HT	160.7 (103.7)	144.3 (101.4)	171.2 (117.8)	160.4 (106.9)	171.5 (115.2)	< 0.001
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2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-11

Title: Urinary arsenic metabolites and risk of breast cancer molecular subtypes in northern Mexican women

López-Carrillo L, Gamboa-Loira B and Cebrián ME E. Instituto Nacional de Salud Publica, Cuernavaca, Morelos, Mexico and Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional, Mexico, Ciudad de Mexico, Mexico.

Body: Background/Aim: For many years, the presence of arsenic (As) in drinking water of some areas in Northern Mexico has been a major concern. As levels have ranged from 71 to 600 µg/L. We have recently reported that women residing in Northern Mexico, with higher urinary monomethyl arsenic acid percentage (%MMA) had an increased breast cancer (BC) risk (MMA% ORQ5vs.Q1=2.63; 95%CI: 1.89, 3.66; p for trend <0.001) (López-Carrillo *et al.*, 214).

Breast tumor subtypes with distinctive biology and treatment responses are defined by the immunohistochemical expression of estrogen and progesterone receptors (HR) and human epidermal growth factor receptor 2 (HER2). Epidemiological evidence suggests that BC risk factors (i.e. breastfeeding, parity, menarche, etc.) may vary by tumor pathology. We assessed if %MMA is associated with a particular BC molecular subtype.

Methods: A population based case–control study was performed from 2007 to 2011 in five states of Northern Mexico. Incident cases were identified from main public tertiary hospitals in the study area. A total of 499 patients with histopathologically confirmed BC and molecular subtype status available information were identified. Controls were healthy women, with no history of cancer, matched 1:1 by age.

Tumor marker information for estrogen receptor (ER), progesterone receptor (PR) and HER2 was obtained from medical records. ER+ and PR+ was based on $\geq 1\%$ cell staining. HER2+ status was determined when at least 30% of protein overexpression was observed in the tumor cell. Cases were assigned to one of three tumor marker categories: HR+ (ER+ and/or PR+ and HER2-), HER+ (regardless of ER or PR status) and TNBC (ER-, PR-, and HER2-).

Urinary concentrations (µg/L) of species As³⁺, As⁵⁺, MMA⁵⁺, DMA⁵⁺, and arsenobetaine (AsB) were determined by high-performance liquid chromatography ICP-MS. Detection limits were As³⁺: 0.12; As⁵⁺: 0.20; MMA⁵⁺: 0.12; DMA⁵⁺: 0.08; AsB: 0.08. Results were reported by g of creatinine. Total As was calculated by using both the sum of iAs, MMA, DMA plus or minus AsB concentration (TAs or TAs-AsB, respectively). iAs was calculated as the sum of trivalent (As³⁺) and pentavalent (As⁵⁺). %iAs, %MMA and %DMA species were estimated using TAs-AsB as denominator.

Using logistic regression models, the associations between BC molecular subtypes and MMA% and DMA% was estimated according to the observed quintile distribution among controls. Based on the median value of each quintile a lineal trend was assessed accordingly.

Results. The mean age of cases and controls was around 54 years with 51 years of residence in the study zone. In the entire population, TAs ranged from 0.47 to 303.29 µg/L (0.45 to 1683.83 µg/g creatinine). HR+ BC patients were 57%, HER2 23% and TNBC 20%. These figures are similar to those reported among other groups of Mexican cancer patients. After controlling by key BC factors, %MMA remained as a significant risk factor for BC only for HR+. HER2+ and TNBC tumors were not significantly associated with urinary As metabolites.

Conclusion: Our results suggest that increased BC risk related to As exposure is not mediated by HER2 expression. This is the first report on As exposure and breast cancer subtypes which requires further confirmation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-12

Title: HER2 status remains the primary predictor of improved survival in patients with BCBM over the past 2 decades (1996-2015)

Narloch JL L, Harnden K, Broadwater G, Bercedis PL L, Terry H, John K, Peter FE E, Grace KJ J and Kimberly BL L. Clinical Research Training Program, Duke University Medical Center, Durham, NC; Duke University Medical Center, Durham, NC; Duke Cancer Institute, Durham, NC; Duke University Medical Center, Durham, NC and Duke University Medical Center, Durham, NC.

Body: BACKGROUND: Brain metastasis is a complication in advanced breast cancer (ABC) and is associated with poor prognosis. Incidence of breast cancer brain metastasis (BCBM) is increasing with advances in therapy, allowing patients to survive long enough to develop CNS metastasis. Improved outcomes have been documented in ABC over the past decades, largely related to the use of trastuzumab in HER2+ ABC. However, it remains unclear whether survival has improved in HER2-ABC in patients with BCBM. This study asks: has the improvement in systemic and radiotherapies for HER2- breast cancer impacted survival in patients with breast cancer brain metastasis.

OBJECTIVES: 1) To estimate whether date of BCBM diagnosis is associated with overall survival (OS) in patients diagnosed between 1996-2015. 2) To estimate whether OS of this patient population depends upon other demographic and clinical factors.

METHODS: This is a retrospective chart review of patients with diagnosis of BCBM between 1996-2015. Data collection includes: age at BCBM diagnosis, ethnicity, ER/PR/HER2 status, date of BCBM diagnosis, date of primary breast cancer diagnosis, date of death/last clinical follow-up, and treatment. Kaplan-Meier analysis and the log-rank test compared OS (time from diagnosis of BCBM until death or last clinical FU) between groups diagnosed in 5-year cohorts (1996-2000, 2001-2005, 2006-2010, 2011-2015). A univariate proportional hazards model was used to regress OS on date of diagnosis. A multivariate proportional hazards model was used which included the subset of patients diagnosed with BCBM in 2001 and later. This model adjusted for additional factors: race, time to development of BCBM diagnosis, age at the time of BCBM diagnosis, year of diagnosis as a continuous factor, ER, PR, while testing the significance of HER2 status. A p-value < 0.05 was significant.

RESULTS: A total of 165 patients with BCBM were included in this analysis, with a median age of 53.8 (SD 13.0) at time of BCBM diagnosis. Most patients were Caucasian (66%; 109/165) or African-American (29%; 48/165). Although statistical significance was not attained, greater median overall survival was seen for patients diagnosed with BCBM in more recent 5-year cohorts (2011-2016, 9.5 months; 2006-2010, 8 months) than patients in older cohorts (2001-2006, 3.6 months; 1996-2000, 5.3 months), p=0.3. Date of diagnosis of BCBM as a continuous variable is predictive of overall survival (HR 0.83 [95% CI: 0.71-0.97] comparing 5-year intervals, p=0.016). After adjusting for the covariates listed above, HER2 positive status is predictive of overall survival (HR 0.34 [95% CI: 0.34-0.56]; p<0.0001).

CONCLUSIONS: While survival has improved by 5.9 months over the past two decades, it remains highly dependent on HER2 status. Novel therapies for BCBM are greatly needed for ER+ and triple negative subtypes. Final results will include an expanded analysis to incorporate additional cases and three other categorical covariates measured during follow-up: whether the patient received radiotherapy, surgery, and/or medical therapy after diagnosis of brain metastases.

GRANT FUNDING: TL-1 CTSA Pre-Doctoral Training Grant (5TL1TR001116-03).

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-13

Title: Phyllodes tumours of the breast: The British Columbia cancer agency experience

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Body: Background

Phyllodes tumours (PT) of the breast are uncommon fibroepithelial lesions for which optimal management remains unclear. This population-based study reports treatment and outcomes for patients with PT and evaluates characteristics that influence outcome.

Methods

Data were analysed on 183 patients with newly diagnosed PT referred to the BC Cancer Agency from 1999-2014. Follow-up was obtained from chart and death records and letters to general practitioners. Five-year Kaplan-Meier (KM) local recurrence (LR) and survival were compared between cohorts with benign (n=83), borderline (n=50) and malignant PT (n=49) histology. Subtype was unknown in 1 patient who did not receive surgery due to severe comorbidity. Univariate analysis was performed using Cox regression modeling.

Results

Median follow-up was 65 (range 0.5-197) months. Median age was 48 (range 14–87) years. Median tumour size was 4 (range 1-23) cm. Heterologous sarcomatous differentiation was seen in 15 and malignant epithelial transformation in 11 patients. Local excision was performed in 163 (89%) and mastectomy in 19 (10%) patients. Margin status after local excision were: negative (>1mm, n=121, 74%), close (\leq 1mm, n=21, 13%), positive (tumour touching ink, n=20, 12%), or unknown (n=1, 1%). Margin status after mastectomy were negative (n=14, 74%) or close (n=5, 26%). Tumour borders were pushing (n=62, 34%), intermediate (n=22, 12%), infiltrative (n=38, 21%) or unknown (n=60, 33%). Eleven patients with malignant PT received radiation therapy as part of initial treatment. In these cases median tumour size was 8 cm and heterologous sarcomatous differentiation was present in 46% compared to 4 cm and 16% in malignant cases who did not receive RT.

LR occurred in 16 cases (5 benign, 4 borderline and 7 malignant). Distant metastases (DM) occurred in 7 patients with malignant PT leading to 6 cause specific deaths.

Five-year KM outcomes among women with benign, borderline, and malignant PT were: LR 6% vs 9% vs 21%, $P=0.131$; overall survival 96% vs 100% vs 82%, $P=0.002$; and disease free survival 94% vs 91% vs 67%, $P<0.001$. DM-free and cause specific survival at 5 years for malignant cases were 82% and 88% respectively.

Five-year KM LR among women with negative vs close vs positive margins were 8% vs 6% vs 37%, $P<0.001$. Corresponding rates for intermediate vs pushing vs infiltrative borders were 6% vs 6% vs 33%, $P=0.006$.

On univariate analyses, large tumour size, postmenopausal status, malignant classification, necrosis, positive margins, and infiltrative borders were factors associated with increased risk of any type of relapse (all $P\leq 0.014$). Positive margins and infiltrative tumour border were associated with increased LR (all $P\leq 0.006$), and the latter remained significant in exploratory analyses after adjusting for margin status and PT classification. Advanced age at diagnosis and large tumour size were predictors of DM (all $P\leq 0.001$).

Conclusion

In this population-based series, 5-year outcomes among women with PT are comparable to those reported in the literature. Exploratory analysis suggests that infiltrative tumour borders may be used in conjunction with margin status to assess LR risk. While close margin was not associated with increased LR, re-excision is warranted for cases with positive margins.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-14

Title: Synchronous bilateral breast cancer compared to unilateral breast cancer: A population based study

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Body: Introduction: The clinical presentation and outcome of synchronous bilateral invasive breast cancer compared to unilateral breast cancer is not well studied. In this study, we present a population based comparison of tumor characteristics and survival between invasive unilateral and bilateral breast cancer.

Methods: Data from National Cancer Institute- Surveillance, Epidemiology and End Results (SEER) was analyzed to identify women who had a diagnosis of invasive ductal carcinoma (ICD-O-3: 8500/3) between the years 2000 and 2013. Women who were diagnosed with an invasive contralateral breast cancer within two months of initial diagnosis were classified under synchronous bilateral breast cancer group. Women who developed a contralateral breast cancer between three to twelve months of diagnosis were excluded. All women who did not develop a second breast cancer, or developed it after twelve months of initial diagnosis were classified under unilateral breast cancer group. Patients with an in-situ breast cancer diagnosis in the contralateral breast within twelve months of diagnosis of invasive ductal carcinoma were excluded. Patients with metastatic disease and unknown stage at presentation were excluded. Tumor characteristics and survival were compared between the two groups using the index case only. Mann-Whitney U test was used for continuous variables while Chi-square test was used for categorical variables. Kaplan-Meier curves and Cox proportional hazard regression models were used to compare survival. All data analysis was performed using SPSS 21.

Results: A total of 414,766 patients met our inclusion criteria. Of these, 3,590 patients were in the synchronous bilateral breast cancer group, while 411,176 were in the unilateral breast cancer group. Compared to unilateral breast cancer patients, bilateral breast cancer patients were slightly older (62 Vs 59, $p<0.05$) and were more likely to have higher T, N or overall stage at presentation. Their tumors were also more likely to be estrogen or progesterone receptor positive ($p<0.05$). Patients with bilateral breast cancer also had higher rates of mastectomy (63.3% Vs 39.1%, $p<0.05$).

Patients with bilateral breast cancers had a significantly worse survival compared to patients with unilateral breast cancer (Table 1). In a multivariate Cox proportional hazard regression, adjusting for age at diagnosis, T-Stage, N-Stage, overall stage, hormone receptor status, type of surgical treatment and radiation therapy, bilateral breast cancers had a mortality hazard ratio of 1.48 (95% CI: 1.35 – 1.62, $p<0.001$) compared to unilateral breast cancers.

Conclusions: Our study demonstrates that bilateral breast cancer has a worse prognosis compared to invasive unilateral breast cancer. These findings have important implications for patients, and contribute to our understanding of the unique outcomes of bilateral breast cancer which will guide treatment and follow up.

Table 1: Survival of unilateral vs bilateral breast cancer

	Unilateral breast cancer	Bilateral breast cancer
5-year cause specific survival	91.7%	86.5%
10-year cause specific survival	86.1%	77.7%

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-15

Title: Real-world treatment patterns and survival among triple negative breast cancer patients versus patients with other breast cancer subtypes in early stage breast cancer

DaCosta Byfield S, Abushamaa AM M, Becker LK K, Shepherd SP P, Ricker JL L and Bonnet P. Optum, MN and Abbvie, IL.

Body: Background: It is generally accepted that outcomes of breast cancer (BC) patients (pts) differ by tumor characteristics, but relatively few studies have examined treatment patterns and outcomes in early stage disease by hormone receptor (HR) and HER2 status.

Methods: This retrospective study used physician-reported clinical information (date and stage at diagnosis, HER2 and HR status) for commercially insured BC pts from the Optum Oncology Management Registry linked with the Optum Research Database (medical and pharmacy claims from a national US health plan) and death data from Social Security Administration files from 01/2008 to 6/2014. Pts diagnosed with early stage breast cancer (ESBC) were examined. The index date was diagnosis of BC. Adult pts (≥ 18 yrs old) had to have known HR and HER2 status, continuous enrollment in the health plan from initial diagnosis for ≥ 6 months, surgery and systemic cancer therapy. Neo-adjuvant and adjuvant therapy (initial phase of care) based on timing of claims for NCCN-recommended therapy were examined, as well as survival and development of metastasis (based on claims with ICD-9 codes 196.0x, 196.2x, 196.5x-199.0x) after initial phase of care during the study period. Differences across the HR/HER2 subtypes were examined using t-test for continuous variables and chi-square test for categorical variables.

Results: Among 6,881 ESBC pts identified, 15% (n=1038) were TNBC pts, 65% HR+/HER2-, 14% HR+/HER2+ and 6% HR-/HER2+. Mean (median) age was 52 (53) years. Treatment patterns and outcomes vary by tumor subtype (Table).

	HR-/HER2-	HR+/HER2-	HR-/HER2+	HR+/HER2+
	N=1038	N=4452	N=401	N=990
Length of follow-up, mths, mean(SD)*, median	27 (16), 23	28 (16), 25	29 (16), 25	27 (15), 24
Length of initial phase of care – mths, mean(SD)*, media	10 (7), 9	22 (15), 19	15 (9), 14	23 (14), 19
Pts with systemic neo-adjuvant therapy N (%)*	303 (29)	531 (12)	140 (35)	216 (22)
Most common neo-adjuvant regimens (%)	Paclitaxel, cyclophosphamide, doxorubicin (45%)	Paclitaxel, cyclophosphamide, doxorubicin (31%)	Docetaxel, carboplatin, trastuzumab (44%)	Docetaxel, carboplatin, trastuzumab (31%)
Pts with systemic adjuvant therapy N (%)*	740 (71)	4065 (91)	366 (91)	935 (94)
Most common adjuvant regimens (%)	Paclitaxel, cyclophosphamide, doxorubicin (30%)	Tamoxifen (19%)	Docetaxel, carboplatin, trastuzumab (30%)	Docetaxel, carboplatin, trastuzumab, tamoxifen (9%)
Evidence of distant metastasis N (%)*	78 (7.5)	76 (1.7)	17 (4.2)	15 (1.5)
Mortality N (%)*	23(2.2)	23 (0.5)	1 (0.3)	3 (0.3)

*p<0.05

Conclusions: The most common neo-adjuvant and adjuvant chemotherapy agents received were similar across HER2 subtypes and regimens for HR+ and HER2+ patients also included hormonal or HER2 targeted agents. TNBC pts had the shortest length of initial phase of care (mean 10 mths), though this cohort had the second highest percentage of patients receiving systemic neo-adjuvant therapy. Development of distant metastasis and mortality during the study period were highest for the HR-/HER2- patients, at 7.5% and 2.2% respectively. Development of novel targets and therapies for TNBC may significantly benefit patients with this BC subtype, in both neo-adjuvant and adjuvant setting to improve outcomes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-16

Title: Breast cancer in the Bahamas: Revisiting the adequacy of national screening guidelines

Deleveaux S, Curling D and Francis W. University of the West Indies School of Clinical Medicine and Research, Nassau, Bahamas; Princess Margaret Hospital, Nassau, Bahamas and Princess Margaret Hospital, Nassau, Bahamas.

Body: Background: The Bahamas is an island nation shown to have a high incidence of early onset breast cancer. In addition, recent research has revealed that the Bahamian population has a high prevalence of a founder mutation to the BRCA1 gene. Despite the population differences between the Bahamas and the United States, the screening guidelines for breast cancer in the Bahamas reflect those of the United States. This study provides data that suggests that the current breast cancer screening guidelines in the Bahamas do not capture cases in the age groups most at risk and are in need of revision.

Methods: We performed a retrospective review of all cases submitted to the Princess Margaret Hospital cancer registry from 1998-2012. We divided the patients into 10 year age cohorts and determined the frequency of breast cancer in each group. For comparison, data for the United States was obtained using the Surveillance, Epidemiology, and End Results (SEER) 18 registries, including all cases of breast cancer from 1998-2012 further stratified into age groups and ethnicity.

Results: The average age of diagnosis of breast cancer in the Bahamas from 1998-2012 was 54 years, compared to an average age of diagnosis in the United States overall population of 61 years and among the black American ethnicity, 58 years (2008-2012 data). The age group with the highest percentage of incidence in the Bahamas is the 40-49 age group consisting of 27% of those diagnosed from 1998-2012. During the same time period, the age group with the highest incidence of breast cancer in the United States was the 50-59 age group in the overall population (24.8%), as well as in the black population (26.7%). By beginning screening at age 40, according to screening guidelines during the study period, the United States was able to capture 95.3% of breast cancer cases in the overall population and 92.8% of breast cancer cases in the black population. Using similar guidelines, the Bahamas captured only 83.6% of breast cancer cases. For comparison, if screening began at age 30 in the Bahamas during the study period, 97% of breast cancer cases would have been captured.

Conclusions: The data in this study demonstrates significant differences in the incidence of breast cancer between age group cohorts in the population of the Bahamas compared to the population of the United States. Furthermore, it suggests that the screening guidelines in place in the Bahamas may be insufficient at capturing an adequate percentage of the population at risk for breast cancer. A significant increase in capture of those with breast cancer in the Bahamas may be achieved by reducing the age to begin screening below the age of 40.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-17

Title: Breast cancer in octogenarians: A community hospital experience

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Body: Background : Breast cancer incidence increases with age. However Elderly patients are undertreated and underdiagnosed affecting their overall survival with limited availability of clinical trial data

Patients and Methods : We used the data from our cancer registry to evaluate the cases of breast cancer diagnosed between the years 2005-2010 with follow up data available to date to examine the method of diagnosis(mammogram vs palpable abnormality), Age at diagnosis, Stage at diagnosis, Type of surgery (BCT Vs Mastectomy), Receptor status(ER/PR/Her 2 neu) and treatment's received (Hormonal therapy , Radiation therapy and chemotherapy) and average survival after the diagnosis of breast cancer. Data regarding the causality of death was not readily available as part of the registry data.

Results : A total of 495 breast cancer cases diagnosed in the years between 2005-2010 were identified ., Median age at diagnosis was 85 with ages ranging from 81-102 .Out of the 495 patients 55 % were diagnosed with a mammogram and 41 % by palpable abnormality. Patients were diagnosed at Stage 0,1,2,3,4 (12%,45 %,7% ,3 %. 55% underwent Lumpectomy and 31 % underwent Mastectomy .82 % and 18 % ER negative. Her 2 data was only available in 59 patients, out of those tested only 4 Her 2 positive. , Radiation(34 %) /Chemo(6 %)/ Hormone(34%) ,Radiation/Chemo 2 % ,Radiation/Hormone 18%, Chemo/Hormon 2% , 41 % didn't proceed with any treatments.Median survival was 54 months and Average survival was 56 months. Median survival 54 months and average survival was 56 months, With 203 patients alive at the end of 5 years with mortality of about 62 %. However we do not have the data of causality of death in these patient .

Comparison between Age Groups : See table below

Comparison of data b/n Age groups

Age at Diagnosis	80-84	85-89	90 +
No of patients	244	192	56
Mammogram	138	105	32
Palpable Lump	94	79	24
Lumpectomy	157	67	24
Mastectomy	80	30	26
Average Survival in months	84.5	48	48.5

Conclusion: Even though Surgery was performed in most the patients , Only 50 % or lesser number of patients had Adjuvant treatment with Endocrine , Radiation and chemotherapy. Based on the data even though most patient were diagnosed with early stage breast cancer , had significantly poor survival with median survival of about 54 months . With Survival dramatically dropped from 84 months to 48 months in patient 85 + years of age.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-18

Title: Treatment patterns and resource utilization among patients with HR+/HER2– metastatic breast cancer in a privately insured US population

Goyal RK K, Carter GC C, Nagar SN N, Smyth EN N, Price GL L, Huang Y-J, Bromund JL L, Li L, Schilder JM M, Davis KL L and Kaye JA A. RTI Health Solutions, Research Triangle Park, NC; Eli Lilly and Company, Indianapolis, IN and RTI Health Solutions, Waltham, MA.

Body: Background

Hormone receptor positive (HR+)/HER2– tumors are the most common subtype among patients with metastatic breast cancer (MBC). Several newer therapeutic options have become available over the last decade, but little is known about the real-world treatment patterns and health care resource use (HCRU) in privately insured women with HR+/HER2– MBC.

Methods

An analysis of Truven MarketScan databases containing medical and drug utilization and productivity data from nearly 350 US payers was conducted. Patients aged 18-64 years with an ICD-9 diagnosis code of breast cancer along with ≥ 2 claims for secondary malignancy between 2007 and 2013 were selected. HR+/HER2– patients were identified based on receipt of endocrine therapy (ET) and absence of HER2-targeted therapies. Use of cancer-directed treatments following MBC diagnosis was analyzed. Treatment characteristics were examined by line of therapy (LOT). Average monthly all-cause and MBC-related HCRU were descriptively assessed.

Results

A total of 5,563 women with HR+/HER2– MBC (mean [SD] age, 54 [7.8] yrs) met the selection criteria. Overall, 97% of the total sample received ≥ 1 cancer-directed treatment. The most common treatment was ET (85%), followed by chemotherapy (CT) (70%), radiation (62%), and surgery (11%). Treatment patterns for CT alone and ET alone, including the top regimens by LOT, are presented in Table 1. Among those receiving a second LOT, nearly 44% switched to CT in the second line after having received ET alone in the first line. During the study follow-up, 56% of patients had ≥ 1 all-cause inpatient admission, 49% had ≥ 1 all-cause emergency department visit, and 9% had a hospice admission.

Table 1. Treatment patterns by LOT in patients with HR+/HER2- MBC

Line 1		Line 2		Line 3		Line 4	
n=5,179 (93%)*		n=2,900 (52%)*		n=1,608 (29%)*		n=882 (16%)*	
	n (%)		n (%)		n (%)		n (%)
ET Alone	3265 (63)	ET Alone	1468 (51)	ET Alone	534 (33)	ET Alone	217 (25)
Anastrozole	895 (27)	Fulvestrant	354 (24)	Fulvestrant	138 (26)	Fulvestrant	65 (30)
Letrozole	782 (24)	Tamoxifen	258 (18)	Exemestane	89 (17)	Exemestane	44 (20)
Tamoxifen	577 (18)	Exemestane	239 (16)	Letrozole	82 (15)	Tamoxifen	25 (12)
Fulvestrant	428 (13)	Anastrozole	239 (16)	Tamoxifen	82 (15)	Letrozole	20 (9)
Exemestane	299 (9)	Letrozole	197 (13)	Anastrozole	65 (12)	Anastrozole	14 (6)
CT Alone	1533 (30)	CT Alone	1057 (36)	CT Alone	818 (51)	CT Alone	505 (57)
Paclitaxel	413 (27)	Capecitabine	331 (31)	Capecitabine	265 (32)	Capecitabine	140 (28)
Capecitabine	286 (19)	Paclitaxel	224 (21)	Paclitaxel	156 (19)	Paclitaxel	93 (18)
Cyclophosphamide-Doxorubicin → Taxane	93 (6)	Gemcitabine	63 (6)	Gemcitabine	70 (9)	Vinorelbine	55 (11)
Cyclophosphamide-Docetaxel	82 (5)	Docetaxel	46 (4)	Vinorelbine	54 (7)	Gemcitabine	52 (10)

Carboplatin-Paclitaxel	77 (5)	Vinorelbine	46 (4)	Doxorubicin	45 (6)	Doxorubicin	34 (7)
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*Out of total 5,563 patients. Only top CT and ET regimens are listed.

Conclusions

A substantial decrease in the use of ET, with simultaneous increase in the use of CT, was observed as patients progressed to subsequent LOTs. Nearly half of those receiving ET alone in the first LOT switched to CT in the second LOT, suggesting a need for more effective non-CT treatments to bridge unmet therapeutic needs in this patient population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-19

Title: Treatment patterns and resource utilization among elderly Medicare patients with HR+/HER2- metastatic breast cancer

Goyal RK K, Carter GC C, Nagar SN N, Smyth EN N, Price GL L, Huang Y-J, Bromund JL L, Li L, Schilder JM M, Davis KL L and Kaye JA A. RTI Health Solutions, Research Triangle Park, NC; Eli Lilly and Company, Indianapolis, IN and RTI Health Solutions, Waltham, MA.

Body: Background

Therapeutic advances in metastatic breast cancer (MBC) over the last decade have led to several novel agents for the treatment of patients with hormone receptor positive (HR+)/HER2- MBC. However, current literature has little data on real-world treatment patterns and health care resource use, particularly among elderly women with HR+/HER2- MBC in the United States Medicare population.

Methods

A retrospective analysis of patients aged ≥ 66 years diagnosed with MBC during 2007 to 2011 was conducted using the SEER-Medicare database. Patients' HR and HER2 status was obtained from the SEER registry data. For patients with no HER2 data available, HER2- disease was determined based on the absence of HER2-targeted therapies within 12 months of diagnosis. Health care utilization and treatment patterns after MBC diagnosis were examined. Use of cancer-directed therapies, including chemotherapy (CT) and endocrine therapy (ET), were descriptively analyzed by line of therapy (LOT).

Results

A total of 3,622 women with HR+/HER2- MBC (mean [SD] age, 77 [7.3] years) were included. Over 90% of women received ≥ 1 cancer-directed treatment after MBC diagnosis, with ET being the most common (77%), followed by CT (50%), radiation (48%), and surgery (19%). Treatment with ET alone trended downward across LOTs, from 74% in the first LOT to 36% in the fourth LOT, with a corresponding increase in treatment with CT alone from 21% to 46% (Table 1). Among those receiving a second LOT, nearly 26% switched to CT in the second line after having received ET alone in the first line.

Table 1. Pharmaceutical treatment patterns by line of therapy among patients diagnosed with HR+/HER2- MBC (n = 3622)

First-Line		Second-Line		Third-Line		Fourth-Line	
N = 2,981 (82%)*		N = 1,449 (40%)*		N = 750 (21%)*		N = 356 (10%)*	
	n (%)		n (%)		n (%)		n (%)
ET Alone	2215 (74)	ET Alone	973 (67)	ET Alone	381 (51)	ET Alone	127 (36)
Anastrozole	893 (40)	Fulvestrant	282 (29)	Fulvestrant	99 (26)	Fulvestrant	38 (30)
Letrozole	602 (27)	Exemestane	190 (20)	Exemestane	76 (20)	Tamoxifen	27 (21)
Tamoxifen	253 (11)	Anastrozole	162 (17)	Tamoxifen	71 (19)	Exemestane	25 (20)
Fulvestrant	243 (11)	Tamoxifen	152 (16)	Anastrozole	46 (12)	Anastrozole	13 (10)
Exemestane	156 (7)	Letrozole	107 (11)	Letrozole	38 (10)	Exemestane-Fulvestrant	N/A
CT Alone	639 (21)	CT Alone	336 (23)	CT Alone	264 (35)	CT Alone	165 (46)
Paclitaxel	136 (21)	Paclitaxel	76 (23)	Paclitaxel	78 (30)	Paclitaxel	39 (24)

Cyclophosphamide-Docetaxel	72 (11)	Gemcitabine	57 (17)	Gemcitabine	46 (17)	Gemcitabine	32 (19)
Cyclophosphamide-Doxorubicin → Taxane	69 (11)	Docetaxel	28 (8)	Vinorelbine	31 (12)	Vinorelbine	21 (13)
Carboplatin-Paclitaxel	43 (7)	Vinorelbine	27 (8)	Docetaxel	22 (8)	Doxorubicin	17 (10)
Docetaxel	39 (6)	Doxorubicin	21 (6)	Doxorubicin	21 (8)	Docetaxel	N/A

N/A = not available (in accordance with the SEER-Medicare data use agreement, data for categories with cell size less than 11 are suppressed). *Out of total 3,622 patients. Note: Percentages do not add up to 100% as only the top CT and ET regimens are listed.

Conclusions

ET was the most common first-line treatment for elderly women with HR+/HER2- MBC in this study period. However, as patients progressed from first to fourth LOT, the proportion of patients treated with ET decreased substantially.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-20

Title: A real-world evidence study to define the prevalence of endocrine therapy-naïve hormone receptor-positive locally advanced or metastatic breast cancer in the US

Nunes AP P, Green E, Dalvi T, Lewis J, Jones N and Seeger JD D. Optum Epidemiology, Waltham, MA; MedImmune, Gaithersburg, MD and AstraZeneca, Cambridge, United Kingdom.

Body: BACKGROUND

The recently completed FALCON trial (NCT01602380) compared the efficacy of the selective estrogen receptor degrader (SERD) fulvestrant 500 mg with anastrozole in postmenopausal women with hormone receptor (HR)-positive locally advanced or metastatic breast cancer (LA/MBC) who had received no prior endocrine therapy (ET). To better understand the size of the US population to which the results of the FALCON trial are applicable, this study estimated the proportion of postmenopausal patients with HR-positive, human epidermal growth factor receptor (HER)2-negative LA/MBC who had received no prior ET, using data from a US medical record database.

METHODS

This observational study retrospectively analyzed data from the Optum Electronic Health Record Database, obtained from provider groups across the US. Women over 40 years of age with breast cancer diagnoses (January 2008–March 2015) were included, provided they had at least 12 months of recorded medical history prior to index, and at least one recorded physician office visit in that time. Incident (newly diagnosed) and prevalent (previous diagnosis of early or advanced breast cancer) cases were identified. Free-text clinical notes were reviewed using natural language processing to identify a target patient population of postmenopausal women with HR-positive, HER2-negative LA/MBC who had received no prior ET (similar to the FALCON entry criteria); additionally, diagnostic codes or treatment history were also used to identify HR status in the absence of confirmation from the free-text notes. The results of this analysis were extrapolated to estimate the size of the target population at a national level, using statistics from the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) database. Results are presented descriptively.

RESULTS

Overall, 63,962 women with breast cancer were identified, of whom 11,831 had discernible information on menopausal status, HER2 status, HR status, and disease stage. Of these, 1,923 patients were identified with postmenopausal, HR-positive, HER2-negative (or unknown) LA/MBC. Within this population of patients, 70.7% (1,360/1,923) had not previously received ET (88.5% [920/1,040] incident cases; 49.8% [440/883] prevalent cases), representing 11.5% (1,360/11,831) of the total breast cancer population with known menopausal status, HER2 status, HR status, and cancer disease stage information. The proportion of patients with postmenopausal, HR-positive, HER2-negative LA/MBC who had received no prior ET in this sample was extrapolated using US national estimates of the size of the postmenopausal, HR-positive, HER2-negative LA/MBC population taken from SEER. This approach suggests a 5-year limited-duration prevalence of postmenopausal patients with HR-positive, HER2-negative LA/MBC who have received no prior ET of approximately 50,000 cases and an annual incidence of about 15,000 patients.

CONCLUSIONS

These real-world data provide an estimate of the number of postmenopausal patients with HR-positive, HER2-negative, LA/MBC in the US who have not previously received ET. This population corresponds to the recently completed Phase 3 FALCON trial of fulvestrant compared with anastrozole.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-21

Title: The effect of family history on screening procedures and prognosis in breast cancer patients – Results of a large population-based case–control study on breast cancer

Seiffert K, Thoene K, zu Eulenburg C, Rudolph A, Schmalfeldt B, Flesch-Janys D, Chang-Claude J and Witzel I. University Medical Center Hamburg-Eppendorf, Hamburg, Germany; University Medical Center Hamburg-Eppendorf, Hamburg, Germany; University Medical Center Hamburg-Eppendorf, Hamburg, Germany and German Cancer Research Center (DKFZ), Heidelberg, Germany.

Body: Background:

Patients with a first- or second-degree family history of breast or ovarian cancer participate more frequently in breast cancer screening procedures. A benefit of additional screening visits has been documented in high-risk patients with (e.g. BRCA 1, BRCA 2 gene mutations). However, the potential benefit of additional screening examinations in moderate risk patients (patients with a history of breast cancer in one or two family members) remains unclear.

Methods:

Patients were recruited in 2002–2005 within a large population-based case–control study on breast cancer in Germany (3,813 cases and 7,341 age-matched healthy controls). Univariate and multivariate analyses were performed to evaluate the effect of family history on breast cancer risk, participation in screening procedures, tumor size and prognosis.

Results

A higher breast cancer risk was observed in patients with a positive family history (1 affected family member: OR 1.32, $p < 0.001$). Patients with a 1st degree positive family history received a higher number of mammograms (MG) (>10 MG: 42.7% vs. 24.9%, $p < 0.001$) and showed a higher rate of imaging-detected tumors (mammogram or ultrasound) (45.8% vs. 31.9%, $p < 0.001$) compared to patients without positive family history. Positive family history (OR 1.45 pT1 vs. \geq pT2, $p < 0.001$) and higher number of mammograms (≥ 10 mammograms: OR 2.29 pT1 vs. \geq pT2) were associated with smaller tumor size at initial diagnosis. Additionally, there was a significant association between mammogram regularity (HR 0.72, $p < 0.001$) and imaging-assisted tumor detection (HR 0.66, $p < 0.001$) with improved overall survival. However, no significant association between a positive family history of breast cancer and survival could be seen.

Discussion:

Patients with a family history of breast cancer participated more frequently in screening procedures and had a higher rate of image-detected tumors with smaller size at initial diagnosis, compared to patients without affected family members. Breast cancer screening procedures were significantly associated with improved survival.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-22

Title: Impact of delay neoadjuvant chemotherapy on pathologic complete response in locally advanced breast cancer evidence of the real world in Mexico

Cabrera P, Muñoz W, Gutierrez L, Ramirez MT, Albarado A, Lara F and Mohar A. National Cancer Institute of Mexico, Mexico City, Mexico.

Body: Background:

Several studies suggest that delay on initiation adjuvant systemic therapy has subtype specific effects on survival. Many patients need neoadjuvant chemotherapy (NAC) for locally advanced breast cancer (LABC) in middle income countries. The optimal timing from breast cancer (BC) diagnosis to initiation of NAC is unknown and is not known if this interval impacts in pathological complete response (pCR) and survival.

We evaluated the relationship between pCR (defined as no invasive disease in breast and lymph nodes) and the implications in survival outcomes from LABC diagnosis and initiation of NAC.

Methods:

Data were collected retrospectively from database on National Cancer Institute for locally advanced breast cancer (II-III) treated with NAC within six months of their diagnosis and completed treatment between January 2007 to December 2015. We evaluated data's of 934. Time between biopsy result and start of chemotherapy was calculated. Patients were grouped into those who had pCR vs. no pCR, those who started treatment within 28 days and those who started after 28 days and the expression of hormonal receptors and Her2 by immunohistochemistry. We evaluated the results with the Pearson's χ^2 and Fisher's exact test.

Results:

We evaluated data's of 934 woman. Overall pCR rate for our population was (311) 33%. The patients who initiated their treatment before 28 days the pCR rate was 40% and in the group who initiated NAC after 28 days the pCR was 27% with $p=0.0001$, independently of the immunohistochemistry pattern expression. The median overall survival for the patients who initiated NAC before 28 days is 111 months compare with the patients who initiated NAC after 28 days was 101 months with a $p=0.52$. For disease free survival in patients who began NAC before 28 days and achieved pCR were 92.2 months and for the group after 28 days were 89.9 months ($p=0.033$)

If we divided the group in triple negative patients by immunohistochemistry (183) ER negative, PR negative, HER2 negative, the patients with less than 28 days have 49.5% of pCR and the patients who initiated NAC after 28 days have pCR 37% ($p=0.044$). In the group with triple positive 70 patients (ER positive, PR positive, HER2 positive) those who began the NAC before 28 days achieved pCR 45% and those after 28 days have pCR 39% ($p=0.40$). The patients with ER positive and HER2 negative 475, before 28 days achieved pCR 26% and those after 28 days pCR was 11% ($p=0.0001$). And finally the patients with ER negative and HER2 positive have a pCR of 64% if the NAC initiated before 28 days and the pCR was of 52% if the NAC started after 28 days ($p=0.15$).

Conclusions: As in the adjuvant treatment, the time between biopsy and initiation of neoadjuvant chemotherapy has an impact on the pCR rates and survival of patients with breast cancer locally advanced treated at National Cancer Institute of Mexico in the context of a real world scenario.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-23

Title: The share thoughts on breast cancer study: A Greater Plains collaborative cohort characterization project

Chrischilles EA A, Gryzlak B, McDowell BD D, Connolly DW W and Waitman LR R. University of Iowa, Iowa City, IA and Kansas University Medical Center, Kansas City, KS.

Body: Background. The Greater Plains Collaborative (GPC) is a new clinical data research network in PCORNet, the National Patient-Centered Clinical Research Network sponsored by the Patient-Centered Outcomes Research Institute to conduct patient-centered outcomes research and comparative effectiveness research. The Phase I GPC network brought together a diverse population of 10 million people across 1300 miles covering seven states with a combined area of 679,159 square miles. Using input from community members, breast cancer was selected as a focus for cohort building activities. **Objective.** To test the GPC's integrated data sources, technical components, and governance by demonstrating the ability to select, characterize, and achieve at least a 50% response rate for a cohort of breast cancer patients from across the GPC. **Methods.** Each participating medical center extracted, transformed and loaded North American Association of Central Cancer Registries (NAACCR)-formatted data from their institution's tumor registry into its Informatics for Integrating Biology and the Bedside (i2b2) research warehouse. The GPC i2b2 research warehouse is fully de-identified with re-identification possible when accompanied by an approved IRB protocol. From these data, each medical center selected a cohort of all patients age 18 or older diagnosed with breast cancer between January 2013 and May 2014. De-identified data files were submitted to the GPC Honest Broker who applied eligibility criteria and selected a random sample of 250 eligible patients from each center's file. Eligible patients were women with microscopically-confirmed ductal carcinoma in situ or invasive (but not metastatic) breast cancer diagnosed during the study period. Women who had previously been diagnosed with cancer per tumor registry records were excluded as were women known to be deceased at the time the sample was selected. The sample of patients plus a list of up to 10 replacement patients was provided to each center for re-identification and mailing. Patients participated in designing the study materials. **Results.** IRBs at 7 of 8 collaborating medical centers ceded IRB review to the University of Iowa IRB pursuant to the GPC reliance agreement. Upon patient advice to ensure a representative cohort, a mailed questionnaire with patient incentive was employed. All study materials were mailed in a single packet containing a cover letter from the participating medical center, a 21-page questionnaire, medical record consent and \$10 incentive. Questionnaires were mailed over a six-week period beginning June 19, 2015 and one re-mailing to nonrespondents was conducted 4 weeks after the initial mailing. A total of 1,986 patients were invited and 1,235 (62.2%) responded. Signed consent to obtain information from medical records was obtained for 852 (69%). The survey dataset for consented subjects was linked to the research warehouse to obtain tumor and treatment data and diagnosis and procedure codes from billing sources. **Conclusion.** The GPC's integrated data sources, honest broker-mediated approach to extract data and apply standardized clinical criteria, IRB reliance model, and patient study design partners were implemented successfully.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-24

Title: How do real-world treatment patterns compare to guideline recommendations for first-line metastatic breast cancer patients in US community clinics?

Chu L, Yoo B, Carrigan G, Lai C, Beattie M and Reyes C. Genentech, Inc, South San Francisco, CA.

Body: Background:

Treatment (txt) guidelines are based on trial data from a small minority of patients (pts). Linked electronic health records (EHRs) are a novel approach to examine txt patterns and outcomes in larger and more generalizable populations. Given the increasing importance of real world data and real world outcomes, we utilized linked EHRs from a network of US community clinics to examine how real world txt patterns compare to metastatic breast cancer (mBC) txt guideline recommendations.

Methods:

The Flatiron database provides real world clinical data collected from EHRs used by US cancer care providers. The Flatiron network comprises ~15% of US cancer pts and is geographically and demographically diverse.

Using EHR from Jan 2016 mBC database, we evaluated first-line (1L) txt patterns in mBC by molecular subtype. Pts were selected if they received mBC txt within 60 days of mBC diagnosis between 01Jan2011-31Dec2015, had ≥2 visits within the Flatiron Network on or after 01Jan2013, and were ≥18 years (yrs). Analyses were conducted to describe pt and clinical characteristics and 1L txt by HER2 and/or hormone receptor (HR) status.

Results:

Among 2509 mBC pts identified, 58.9% were HR+/HER2-, 17.7% HER2+, 11.6% HR-/HER2- (triple negative, TNBC), 7.4% HER2 equivocal, and 4.3% 'not done/unknown HER2 status'. Txt patterns in the latter two groups were not analyzed. Selected pt and disease characteristics by subtype are shown in Table 1. Median follow-up since mBC diagnosis was 1.1 yrs (range 0-5 yrs). The 1L mBC txts by subtype are shown in Table 2. Pts with HR+/HER2- subtype were treated primarily with hormonal therapy (68%) and/or chemotherapy (chemo) (35%). Among HER2+ pts, the 1L mBC txt patterns include trastuzumab+pertuzumab with chemo (31%), trastuzumab with chemo (22%), trastuzumab with hormonal therapy (9%), ado-trastuzumab (4%), lapatinib with chemo (3%), and lapatinib with hormonal therapy (1%). For TNBC, the majority received chemo (95%), such as paclitaxel (21%), nab-paclitaxel (13%) and docetaxel (12%).

Conclusion:

This study advances our current understanding of real world 1L patterns of care by molecular subtype among mBC pts and how these compare to guideline recommendations. While the majority of pts are receiving therapy per guidelines, up to 22% of HER2+ of pts are not receiving targeted therapy in 1L mBC

Table 1. Patient and disease characteristics by subtype

N (%)	HR+/HER2- (N=1479)	HER2+ (N=445)	TNBC (N=291)
Age at mBC diagnosis (yrs), median (range)	66 (24-85)	60 (27-85)	60 (33-85)
Sex			
Female	1459 (99)	441 (99)	289 (99)
Race			
White	995 (67)	274 (62)	168 (58)
Black	112 (8)	44 (10)	42 (14)
Asian	19 (1)	14 (3)	5 (2)
Other	179 (12)	50 (11)	36 (12)
Missing	173 (12)	63 (14)	40 (14)
MBC type			
De novo	418 (28)	172 (39)	94 (32)

Recurrent	881 (60)	225 (51)	178 (61)
Unknown	180 (12)	48 (11)	19 (7)
HR Status			
Positive	1479 (100)	300 (67)	--
Negative	--	145 (33)	291 (100)

Table 2. 1L mBC treatments by subtype

N (%)	HR+/HER2- (N=1479)	HER2+ (N=445)	TNBC (N=291)
Any Targeted Therapy*	77 (5)	346 (78)	12 (4)
Any Chemotherapy	521 (35)	283 (64)	276 (95)
Any Hormonal Therapy	1010 (68)	115 (26)	16 (6)

*Targeted therapy includes trastuzumab, pertuzumab, ado-trastuzumab emtansine, lapatinib and bevacizumab

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-25

Title: The association between smoking and breast cancer characteristics and outcome

Goldvaser H, Gal O, Rizel S, Hendler D, Neiman V, Shochat T, Sulkes A, Brenner B and Yerushalmi R. Rabin Medical Center, Petach Tokva, Israel.

Body: Background: Smoking is associated with an increased incidence of hormone receptor positive breast cancer. Data regarding worse breast cancer outcome in smokers are accumulating. Current literature regarding the impact of smoking on breast cancer characteristics is limited. The aim of this study was to evaluate the impact of smoking on the characteristics and outcome of estrogen receptor positive, human epidermal growth factor receptor 2 (HER2) negative early breast cancer.

Methods: This was a retrospective single center study. All patients diagnosed from 4/2005 through 3/2012 and treated in our institute for early, estrogen receptor positive, HER2 negative breast cancer, whose tumors were sent for Oncotype DX analysis were included. Medical records were reviewed for demographics, clinico-pathological parameters, treatment and outcome. Patients were grouped and compared according to smoking history (present or past smokers vs. never smokers) and status (current vs. former and never smokers). Heavy smokers (pack years ≥ 30) were analyzed separately.

Results: A total of 671 patients were included. 28.7% had a history of smoking, 17% were current smokers and 11.5% were heavy smokers. Smoking had no impact on tumor size, nodal involvement and Oncotype DX recurrence score. Angiolymphatic and perineural invasion rates were higher in current smokers than in the rest of the cohort (11% vs. 5.1%, $p=0.023$, 9% vs. 3.45%, $p=0.013$, respectively). Smoking had no other impact regarding histological characteristics. Five-year disease free survival and overall survival rates were 95.7% and 98.5%, respectively. Smoking had no impact on outcome.

Conclusions: In patients with estrogen receptor positive, HER2 negative, early breast cancer, smoking had no clinically significant influence on tumor characteristics and outcome. As the study was limited to a specific subgroup of the breast cancer population in this heterogeneous disease and since smoking is a modifiable risk factor for the disease, further research is required to clarify the possible impact of smoking on breast cancer.

Breast cancer characteristics according to history of smoking, smoking status and pack years

Population (no.)	Mean tumor size, cm (SD)	Macroscopic N+ (%)	Oncotype Dx RS, mean (SD)	Histology, IDC (%)	Angiolymphatic invasion (%)	Perineural invasion (%)	Ki67 (%), mean (SD)
Hx smoking (178)	1.66 (0.9)	10.7	18.9 (9.4)	81	7.7	5.9	15.8 (13.5)
No Hx of smoking (443)	1.7 (0.8)	8.4	19.33 (10.8)	80	5.4	3.8	16 (14.1)
Active smokers (104)	1.76 (1)	9.6	19.7 (9)	82	11	9	16.5 (14.6)
Never/former smokers (513)	1.68 (0.8)	9	19.1 (10.5)	80	5.1	3.5	15.8 (13.7)
PY 0-29 (523)	1.69 (0.8)	9.4	19.2 (10.7)	80	6	4	16.2 (14.2)
PY ≥ 30 (67)	1.67 (1)	9	18.3 (8.8)	85	9	9	13.8 (12.1)

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-26

Title: Treatment patterns for breast cancer brain metastasis

Haraldsson B, Leone JP P, McDowell BD D and Chrischilles EA A. University of Iowa, Iowa City, IA and University of Iowa Hospitals and Clinics, Iowa City, IA.

Body: Background: There is sparse data on how mainstay treatments for brain metastasis (BM) are used in clinical practice and whether their usage has changed over time. Our population-based study presents the rates of surgical resection, radiation therapy, and chemotherapy in a large cohort of women with breast cancer BM. **Methods:** Women diagnosed with breast cancer between 1973 and 2013 were identified from the NCI linkage between the SEER program and Medicare claims data. Identification of BM was via ICD-9 diagnosis codes for secondary CNS malignancy on claims, and was defined as at least one inpatient claim and/or two or more outpatient claims. Treatment was defined as any claim filed with a procedure code for surgical resection of a BM, chemotherapy, and BM-directed radiation therapy. We excluded women without continuous Medicare coverage for a year before to 60 days after their first BM claim and women diagnosed with primary cancer other than breast cancer. **Results:** The proportion of women receiving treatment after BM claim increased from a low of 50.4% in 1992 to 66.8% in 2013 ($p < 0.0001$). Further analysis of this period revealed rates of surgical resection rose from 7.8% to 11.5%, radiation therapy rose from 39.7% to 54.8%, and chemotherapy rose from 11.9% to 22.2% of women ($p < 0.002$). We used adjusted logistic regression to explore associations between patient/tumor characteristics and treatment. Younger age and fewer comorbidities were associated with increased odds of receiving any of the three treatments. Compared with ER-/PR-, a woman whose index cancer was ER+/PR+ had lower odds of receiving any of the three treatments. Women with extracranial metastasis had greater odds of radiation or chemotherapy and lower odds of resection compared to women with BM only. Receiving any care at an Academic Medical Center (AMC) was associated with greater odds of resection, and lower odds of radiation and chemotherapy.

Table 1

	OR; 95% CI		
	Radiation	Resection	Chemotherapy
Age: Per 5 years	0.8; 0.8-0.9	0.8; 0.7-0.9	0.8; 0.7-0.8
Race: Black vs. white	0.7; 0.6-0.9	0.6; 0.5-0.9	0.9; 0.8-1.2
Stage: Distant vs. localized	1.2; 1.0-1.4	0.6; 0.5-0.9	1.1; 0.9-1.3
Comorbidities: ≥ 2 vs. 1	0.7; 0.6-0.8	0.6; 0.4-0.8	0.6; 0.5-0.8
ER/PR: +/+ vs. -/-	0.5; 0.4-0.5	0.6; 0.5-0.7	0.8; 0.7-0.9
Lifetime BC burden: ≥ 2 vs. 1	0.9; 0.8-1.2	1.0; 0.8-1.4	1.0; 0.8-1.3
SEER Registry: 2000+ vs. 1973+	1.1; 0.9-1.2	1.2; 0.9-1.4	0.9; 0.7-1.0
Extracranial metastasis: Y vs. N	2.9; 2.5-3.4	0.8; 0.6-0.9	5.4; 3.8-7.4
Year of BM: Per 5 years	1.1; 1.1-1.2	1.0; 0.9-1.1	1.1; 1.0-1.2
Original reason for Medicare enrollment: Disability or ESRD vs. Age	0.9; 0.7-1.1	0.9; 0.6-1.3	0.6; 0.5-0.8
Marital status: Married vs. not	1.0; 0.9-1.1	1.1; 0.9-1.4	1.2; 1.0-1.3
Interval from BC to BM: ≥ 1 year vs. < 1 year	0.8; 0.7-0.9	1.4; 1.0-1.8	0.8; 0.6-0.9
Rurality of patient's home: Less urban vs. Big metro	1.3; 1.0-1.6	1.0; 0.7-1.5	0.7; 0.5-0.9
Care at an AMC: Y vs. N	0.7; 0.7-0.8	1.5; 1.2-1.8	0.9; 0.7-1.0
Chemotherapy in year before BM: Y vs. N	1.4; 1.2-1.5	0.7; 0.6-0.8	6.0; 5.2-6.9

Conclusions: We found that the proportion of women with breast cancer BM who received treatment has increased over time. Further, logistic regression revealed multiple noteworthy contrasts, including a positive relationship between care at an AMC and

surgical resection, while women who received care at an AMC had lower odds of radiation or chemotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-27

Title: Treatment patterns and clinical outcomes in patients with hormone receptor (HR)+ HER2+ metastatic breast cancer and low vs high levels of HR positivity from the SystHERs Registry

Jahanzeb M, Tripathy D, Rugo H, Swain S, Kaufman PA A, Mayer M, Hurvitz S, O'Shaughnessy J, Mason G, Yardley DA A, Brufsky A, Chu L, Antao V, Beattie M, Yoo B and Cobleigh M. University of Miami Sylvester Comprehensive Cancer Center; University of Texas MD Anderson Cancer Center; University of California San Francisco Helen Diller Family Comprehensive Cancer Center; Washington Cancer Institute, MedStar Washington Hospital Center; Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center; AdvancedBC.org; UCLA Jonsson Comprehensive Cancer Center and Translational Research in Oncology; Baylor Charles A. Sammons Cancer Center, Texas Oncology, US Oncology; Inflammatory Breast Cancer Research Foundation; Sarah Cannon Research Institute and Tennessee Oncology, PLLC; University of Pittsburgh Cancer Institute; Genentech, Inc. and Rush University Medical Center.

Body: Introduction In 2010, the cutoff for HR positivity in breast cancer was established as $\geq 1\%$ of cells staining HR+, previously having varied from 1% to 10%. The impact of this change on treatment patterns and outcomes is poorly understood. SystHERs is a prospective, observational cohort registry of patients (pts) with HER2+ metastatic breast cancer (MBC) that commenced enrollment in 2012. To our knowledge, SystHERs is the largest registry to collect and analyze data for the HER2+ subgroup. We report baseline characteristics, treatment patterns, and early outcomes by %HR+ (1–9% vs 10–100%).

Methods SystHERs enrolled pts aged ≥ 18 years and within 6 months of HER2+ MBC diagnosis. For pts with locally-determined HR+ disease, defined as HR+ in primary or metastatic tissue, %HR+ is the highest percentage of ER+ or PR+ tissue in early breast cancer or MBC. The percentage of ER+ or PR+ cells was not reported for pts considered HR– by the investigator. Median overall survival (OS; Kaplan–Meier) and hazard ratios (Cox regression) were estimated.

Results As of Feb 1, 2016, data were available for 872 eligible pts with known HR status, of whom 608 (70%) had HR+ disease. Of the 608 pts, 53 (9%) had 1–9%HR+ and 496 (82%) had 10–100%HR+; %HR+ was not reported for 59 pts. Baseline characteristics were similar between %HR+ subgroups (Table 1).

As shown in Table 2, the 1–9%HR+ subgroup was less likely to receive first-line hormonal therapy (26%) than the 10–100%HR+ subgroup (56%). 87% and 79% of pts received chemotherapy, respectively.

Median time from MBC diagnosis was 16.5 months (range, 0.4–49.4 months). Median OS was not reached at the data cutoff. The number of deaths was 13 (25%) in the 1–9%HR+ subgroup, and 68 (14%) in the 10–100%HR+ subgroup (log-rank $P=0.025$). The OS hazard ratio (0.514, 95% CI 0.283–0.931) favored the 10–100%HR+ subgroup. OS did not differ significantly between pts with 1–9%HR+ vs HR– disease (log-rank $P=0.582$, hazard ratio 1.185, 95% CI 0.647–2.169).

Table 1. Baseline characteristics

	1-9%HR+ (n=53)	10-100%HR+ (n=496)	HR– (n=264)
Age at MBC diagnosis, median yrs (range)	54 (30–86)	57 (21–86)	55 (28–88)
Race, %			
White	83	83	72
Black	15	13	20
Premenopausal, %	28	25	22
ECOG performance status, %			
0	46	54	44
1	46	39	42
≥ 2	8	7	8
MBC diagnosis type, %			
De novo	40	49	58

Recurrent	60	51	42
Visceral, %*	68	62	75

*Non-hepatic abdominal, ascites, CNS, liver, lung, or pleural effusion sites of metastasis

Table 2. First-line treatment

	1-9%HR+ (n=53)	10-100%HR+ (n=496)	HR- (n=264)
HER2-targeted therapy, %	96	93	91
Chemotherapy, %	87	79	89
Hormonal therapy, %	26	56	4

Conclusions These preliminary observational data suggest potential differences in treatment patterns and survival outcomes in low vs moderate/high HR+ expressers, with the former being less likely to receive hormonal therapy (26% vs 56%). Furthermore, low HR positivity was associated with poorer OS and was similar to OS observed in pts with HR- disease.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-28

Title: Incidence of germline *BRCA1*- and *BRCA2*-mutated breast cancer in the US

Kim R, Peterson A, Isherwood A, Uppal H and Barlev A. Medivation, Inc., San Francisco, CA and Decision Resources Group, London, United Kingdom.

Body: Background: Breast cancer is the most common cancer and second leading cause of cancer death among women in the United States (US).¹ Inherited mutations in germline breast cancer susceptibility gene 1 and 2 (*gBRCAm*) are associated with increased risk of developing cancers, including breast cancer.² No published reports of *gBRCAm* incidence within an unselected US breast cancer population are available based on a comprehensive literature review (CLR). The main objective of this analysis is to estimate the incidence of *gBRCAm* breast cancer in the US.

Methods: For this analysis the Surveillance, Epidemiology, and End Results (SEER) Program 18 registries captured incidence of breast cancer by stage, age and gender.³ The size of the US population was based on United Nation's population projections and standardized to the 2010 population.⁴ Age-specific *gBRCAm* distribution and *gBRCAm*-specific hormonal subtype for estrogen-receptor and progesterone-receptor (ER/PR), and human epidermal growth factor receptor-2 (HER2) estimates were determined from a CLR.⁵⁻⁸ Tumor cells negative for ER/PR and HER2 are referred to as triple-negative breast cancer (TNBC).

Results: In 2016, it is projected that approximately 250,000 individuals will be diagnosed with invasive breast cancer (all genders). Median age range of the population with invasive breast cancer is 65-69 years and 99% are females. Majority (72%) of female invasive breast cancer cases are ER/PR+ whereas 11% of cases are TNBC. Corroborating with current publications, *gBRCAm* is estimated at 5% for individuals less than 50 years old and 1% among all ages. Median age range of the *gBRCAm* cohort is 40-44 years. After applying currently available *gBRCAm* specific literature parameters, the majority (55%) of *gBRCAm* diagnoses are TNBC.

Conclusion: In the US, patients with *gBRCAm* represent a small proportion (1%) of all breast cancer tissues evaluated. Majority of *gBRCAm* patients are diagnosed with TNBC (55%) and are younger (median age range 40-44 years) than overall breast cancer population. Age differences noticed in *gBRCAm* may have been due to disparity in genetic screening practices among breast cancer population in the US rather than a reflection of *gBRCAm* expressions. These estimates of *gBRCAm* incidence are driven by limited reports on an unselected population of breast cancer *gBRCAm* cohort; therefore sensitivity analysis is required to assess the robustness of these estimates.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-29

Title: Trends in age shifting and decreasing mortality of female breast cancer in Taiwan with emerging molecular subset of unmet medical need: A 30-year cohort observation from Taipei Veterans General Hospital

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Body: Background: Over the past thirty years, Taiwan has become a modern Asian country with an increasing incidence of breast cancer. We hypothesized that the epidemiological and demographic features of breast cancer patients in Taiwan have changed by using cohort study based on data collected from past three decades. However, despite improvement in prognosis of most breast cancer subtypes, we found that one subtype of luminal B type her-2 negative did not show significant improvement in overall survival and disease free survival from our cohort comparison.

Methods:Patients with primary breast cancer diagnosed between 1979 and 2010 were identified from the cancer registry database at Taipei Veterans General Hospital, Taiwan. Three periods of time were divided (1979 to 1988, 1989 to 1998, and 1999 to 2010) and compared to determine the epidemiological changes in female breast cancer in Taiwan.

Results:From 1979 to 2010, data from a total of 8417 breast cancer patients were collected. The ratio of breast cancer patients aged ≤ 35 years decreased from 13.3% in 1979 to 1988, to 10.6% in 1989 to 1998, and to 5.2% during 1999 to 2010. In the period of 1979 to 1988, 55.7% of patients were aged ≤ 50 years, and in the period of 1999-2010, only 45.7% of breast cancer patients were aged ≤ 50 yrs. From 1979-1988, ductal carcinoma in situ (DCIS) was rarely diagnosed (0.2%), and the ratio of patients who initially presented with DCIS increased to 2.9% in 1989 to 1998, and to 11.8% in 1999 to 2010. Patients diagnosed with stage I breast cancer also increased from 16.7% in 1979-1988 to 28.4% in 1999-2010. Patients diagnosed and treated in 1999-2010 (5-year overall survival: 81.7%) had a much better outcome than patients treated in 1979-1988 (5-year overall survival: 66.3%). Cohort comparison of survival outcome for breast cancer subtypes in luminal A type, luminal B type Her-2 positive, luminal B type Her-2 negative, Her-2 type, and triple negative type disclosed no improvement in both 5-year survival and 5-year disease free survival outcomes in the subtype of luminal B type Her-2 negative.

Conclusions: The epidemiological features of female breast cancer in Taiwan have changed over the past thirty years. A molecular subtype of luminal B type Her-2 negative was characterized as an emerging group with unmet medical need.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-30

Title: Utilization of oncotype DX for breast cancer in different facility types in Louisiana

Loch M, Li X, Mumphrey B, Garcia A and Wu X-C. LSU Health Science Center, New Orleans, LA.

Body: Background: Oncotype DX, a 21-gene recurrence score, is the most validated prognostic assay that may identify both women most and least likely to benefit from adjuvant chemotherapy. It was added to the ASCO guidelines in 2007 and the NCCN guidelines in 2008 for use in node negative, hormone receptor (HR)+, HER2- breast cancer patients who will undergo 5 years of endocrine therapy. We explored its utilization in different cancer care settings to assess differences in the tool's utilization.

Methods: We analyzed data from the Louisiana Tumor Registry, one of the NCI funded SEER registries for cases of HR+ HER2- localized breast cancer from 2010-2012 and explored differences in utilization of the Oncotype DX by facility type – teaching hospital cancer program (THCP), community hospital comprehensive cancer program (COMP), community hospital cancer program (CHCP), commission on cancer program (COC) and public hospital program. Chemotherapy administration data was collected on the 2011 cases with chemotherapy data collected from a CDC funded special project Enhancing Cancer Registry Data for Comparative Effectiveness Research (CER). Hospital sites were identified by the location where patients underwent surgery. Demographic and clinical factors were adjusted for using multivariate logistic regression.

Results: Of the patients who did have the Oncotype DX, 39.2% were white, 32.3% black. Women over 70 were much less likely (29.3% vs. >45% in women <70) to have the test. Patients who received surgery at public hospitals had the lowest chance (16.7%), whereas those who received surgery at COMP were most likely to have the test (43.1%) and at teaching hospitals, 31.8% had the test. The odds of not using Oncotype DX were significantly higher in public hospitals than THCP even after adjusting for demographic and clinical factors (OR=2.44; 95% CI: 1.39-4.27).

Odds Ratios and 95% CIs for Oncotype DX (No/Yes) in HR+/HER2- localized breast cancer in 2010-2012, Louisiana

Hospital type	#	Crude Odds Ratio and 95% CI	Adjusted Odds Ratio and 95% CI
THCP	422	reference	reference
COMP	489	1.63 (1.24-2.14)	0.56 (0.41-0.76)
CHCP	810	1.35 (1.06-1.74)	0.74 (0.56-0.98)
Public	114	0.43 (0.25-0.73)	2.44 (1.39-4.27)
NonCOC/Non Public	1032	1.37 (1.07-1.74)	0.70 (0.53-0.92)

Conclusions: Patients who had the Oncotype DX performed in our Louisiana database were most likely white, <70 and had their surgeries at COMP. The odds of not having the Oncotype DX performed were significantly higher in public hospitals as compared with teaching hospitals when adjusting for demographic and clinical factors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-08-31

Title: Triple negative breast cancer and factors associated with its treatment in the US – A population study using central cancer registry data

Wu M, Thompson T, Ryerson B, Miller J and Singh S. Centers for Disease Control and Prevention, Atlanta, GA.

Body: Background

Triple receptor-negative breast cancer (TNBC) is characterized by the lack of expression of all the three protein biomarkers, estrogen receptor, progesterone receptor and human epidermal growth factors receptor 2. Large population-based studies on TNBC epidemiology and treatment in the US were generally limited due to lack of routinely data collection on these biomarkers until recent years.

Purpose

This study compared TNBC incidence characteristics to the other subtypes of the breast cancer, examined and documented general treatment status and factors associated with the treatments among TNBC patients in the US.

Methods

We used the NPCR and SEER combined data. All women in the US with a primary invasive breast cancer diagnosed in 2011 and 2012 were included. TNBC age-specific and standard incidence rates, incidence rate ratios by demographic and geographic variables as well as incidence distribution by tumor and clinic characteristics were compared to other subtype of breast cancer. Summary treatment status by staging on TNBC were examined. Regression analysis on factors associated with treatments were also performed.

Results

TNBC indicated demographic, geographic variations, and poor aggressive tumor and clinic-pathologic features compared to other subtypes. 93% women with early stage TNBC had surgery. 31% women with mastectomy chose to undergo contralateral prophylactic mastectomy. Radiation therapy were received or recommended for 64% women with breast conserving surgery (BCS). Chemotherapy were received or recommended for 77% of stage I-IV TNBC women. Among stage I-III patients, sentinel lymph node biopsy was less likely preformed for those aged ≥ 70 , Non-Hispanic African-American, Hispanic, living in Southern Region, and less SES advantage; however, patient's SES status had no impact on surgery type; and there was no association of insurance status and radiation therapy among BCS patients.

Conclusions

Current treatment practice for TNBC in the US is generally concordance with the recommended breast cancer care. However, treatment disparities existed within the limited treatment options, and factors associated with the disparities also varied. More effective treatment options and treatment equality are warranted to improve overall care of this subtype.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-09-01

Title: Plasma osteoprotegerin and the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers

Kotsopoulos J, Oden L, Akbari M, Singer C, Sun P, Salmena L and Narod S. Women's College Research Institute, Women's College Hospital, Toronto, ON, Canada; Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada; Karolinska Institutet, Stockholm, Sweden; Medical University of Vienna, Vienna, Austria and University of Toronto, Toronto, ON, Canada.

Body: Background: There is emerging evidence to suggest that progesterone-mediated upregulation of the receptor activator of nuclear factor κ B (RANK)/RANK ligand (RANKL) signaling pathway plays a critical role in mammary gland epithelial cell proliferation, mammary stem cell expansion and carcinogenesis. Of relevance for women at a high risk of developing breast cancer due to an inherited *BRCA* mutation, are recent findings showing that circulating levels of osteoprotegerin (OPG) (an endogenous decoy receptor for RANKL and thus inhibitor of RANK/RANKL-mediated signaling) are lower in women with a *BRCA1* or *BRCA2* mutation compared to non-carriers. Whether low OPG concentrations contribute to the high breast cancer risk in this population is unknown. If so, a therapeutic intervention that mimics the action of OPG might be used for primary prevention. We evaluated the relationship between plasma OPG and breast cancer risk among women with a *BRCA1/2* mutation in a prospective study.

Methods: Baseline blood samples were available from 199 *BRCA1/2* mutation carriers with no previous history of cancer. Plasma OPG concentrations were measured using a commercial enzyme-linked immunosorbent assay (ELISA) and categorized dichotomously as high vs. low based on the median of the entire cohort. The cumulative incidence of breast cancer by baseline plasma OPG concentration was estimated using Kaplan-Meier survival analysis.

Results: Over a mean follow-up period of 6.6 years (range 0.2-17.6 years), 19 incident cases of primary invasive breast cancer were observed in the cohort. Women who developed breast cancer had significantly lower mean baseline OPG concentrations (83.6 pg/ml [range 4.1-165.6 pg/ml]) compared to the OPG concentrations of women who did not develop breast cancer (107.9 pg/ml [6.7-541.9]) ($P = 0.03$) (Table 1). *BRCA* mutation carriers with low baseline OPG concentrations (< 90 pg/ml) had a significantly higher risk of developing breast cancer compared to those with high baseline OPG concentrations (≥ 90 pg/ml). After ten years of follow-up, the cumulative incidence of breast cancer among women with low OPG concentrations was 23% compared to 7% among women with high OPG concentrations (P -log rank test = 0.01). There was no evidence of effect modification by menopausal status or *BRCA* mutation type.

Plasma OPG concentrations among *BRCA* mutation carriers who did and did not develop breast cancer

Incident Breast Cancer	OPG concentration (range) (pg/ml)	<i>P</i>	
Yes (n = 180)	107.9 (6.7-541.9)		
No (n = 19)	83.6 (4.1-165.6)	0.03	

Conclusions: Our preliminary data suggest that low OPG concentrations are associated with an increased risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers. These data support the potential for targeting of the RANKL pathway as a plausible cancer prevention strategy among women with germline *BRCA* mutations. Additional analyses with a larger sample size are underway.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-09-02

Title: Breast cancer risk prediction using a polygenic risk score in women of African ancestry: Findings from GWAS in breast cancer in the African diaspora

Wang S, Qian F, Zheng Y, Ogundiran T, Ojengbede O, Zheng W, Blot W, Nathanson KL L, Hennis A, Nemesure B, Ambs S, Olopade OI I and Huo D. Center for Clinical Cancer Genetics & Global Health, University of Chicago; University of Chicago; College of Medicine, University of Ibadan; Center for Population and Reproductive Health, College of Medicine, University of Ibadan; Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University; University of Pennsylvania; Chronic Disease Research Centre and Tropical Medicine Research Institute, University of the West Indies; State University of New York at Stony Brook; Laboratory of Human Carcinogenesis, National Cancer Institute and University of Chicago.

Body: Background: Multiple common susceptibility loci for breast cancer (BC) have been identified/**confirmed** in Caucasian women. Combination of these SNPs into a polygenic risk score (PRS) could improve risk stratification and provide guidance for preventive and screening strategies. However, due to differences in allele frequencies of genetic variants, tumor characteristics between women of African and European ancestries, we sought to evaluate the association of PRS with BC in a large consortium of African women.

Methods: The GWAS in BC in the African Diaspora (ROOT consortium) included 3686 participants of African ancestry from Nigeria, USA, and Barbados (1657 cases, 2029 controls). PRS was constructed from the published odds ratios (ORs) from 90 susceptibility loci for BC. Logistic regression was used to examine its association with overall BC risk as well as associations by hormone receptor status, family history and other clinical features.

Results: One unit change in the PRS was associated with an OR of 1.13 (95% CI: 1.01-1.28, $P=0.042$) for overall BC risk, 1.15 (95%CI: 0.95-1.41, $P=0.160$) for ER+ BC risk, and 1.17 (95%CI: 0.95-1.44, $P=0.133$) for ER- BC risk. The ORs for developing BC by percentiles of the PRS, relative to women in the middle quintile, showed weak linear trend

Table 1. Performance of the 90-SNP polygenic risk score (PRS) in the GWAS in Breast Cancer in the African Diaspora

Percentile of PRS (%)	OR (95%CI)		
	Overall BC (n=1657) vs Ctrl (n=2029)	ER+ BC (n=403) vs Ctrl (n=2029)	ER- BC (n=374) vs Ctrl (n=2029)
<5	1.01 (0.73-1.41)	1.32 (0.28-2.13)	0.89 (0.51-1.55)
5-10	0.83 (0.59-1.15)	0.71 (0.40-1.26)	0.63 (0.34-1.17)
10-20	0.74 (0.57-0.95)	0.69 (0.44-1.07)	0.72 (0.46-1.12)
20-40	0.97 (0.79-1.19)	0.80 (0.56-1.14)	0.98 (0.69-1.39)
40-60	1.00 (ref)	1.00(ref)	1.00(ref)
60-80	0.89 (0.73-1.10)	0.94 (0.66-1.33)	0.90 (0.63-1.28)
80-90	1.14 (0.89-1.47)	0.87 (0.56-1.34)	0.90 (0.63-1.28)
90-95	1.14 (0.82-1.58)	1.54 (0.92-2.58)	0.88 (0.48-1.61)
>95	1.10 (0.79-1.52)	1.15 (0.67-1.99)	1.34 (0.79-2.25)

Note: Odds ratios are for different percentiles of the PRS relative to the middle quintile (40% to 60%). Odds ratios were adjusted for study site and the first ten eigenvectors from principal components analysis.

. The discriminative accuracy of the PRS, as measured by the C-statistic, was 0.524 (95% CI: 0.505-0.542) for overall BC, 0.511(95% CI: 0.479-0.543) for ER+ BC, and 0.513 (95% CI: 0.481-0.545) for ER- BC. There was a statistically significant interaction between PRS and age, the association between PRS and overall BC risk were stronger in two age groups (aged <40 years and ≥ 60 years). The PRS was also more strongly associated with PR+ (OR=1.26, 95%CI: 1.01-1.58) compared to PR- (OR=1.08, 95%CI: 0.95-1.44) BC. Association between PRS and BC were similarly non-significant across different strata of family history of BC, BMI, alcohol consumption, oral contraceptive use, menopausal, ER and HER2 status.

Conclusion: BC PRS obtained from prior GWASs conducted in Caucasian women didn't provide a comparable degree of risk

stratification for African Americans. Additional studies are needed to identify SNPs specific to women of African ancestry that could provide improved risk prediction. Further studies can also combine the PRS with lifestyle/environmental factors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-09-03

Title: Associations between breast cancer subtypes and mutations in cancer predisposition genes identified by clinical genetic testing of breast cancer patients

Couch FJ J, Lilyquist J, Na J, Hu C, Polley EC C, Shimelis H, Akinhanmi M, McFarland R, LaDuca H, Goldgar DE E and Dolinsky JS S. Mayo Clinic, Rochester, MN; Ambry Genetics, Aliso Viejo, CA and Univeristy of Utah, Salt Lake, UT.

Body: Clinical genetic testing of individuals with a personal or family history of breast and ovarian cancer using panels for *BRCA1/2* and other candidate cancer predisposition genes has become routine clinical practice. Several of the genes on hereditary cancer testing panels have been strongly associated with specific subtypes of breast cancer. In particular, individuals with germline mutations in *BRCA1* predominantly develop estrogen receptor (ER)-negative and triple negative (TN) (estrogen receptor negative, progesterone receptor negative, HER2 negative) breast tumors. In contrast, *CHEK2* and *ATM* mutations have been associated with ER-positive breast cancer. In this study, associations between mutations in panel genes and breast cancer subtypes were evaluated. A cohort of 60,000 breast cancer patients tested for germline cancer predisposing mutations using hereditary cancer gene panels was utilized. Information on personal and family cancer history, age of diagnosis, tumor pathology, and ethnicity of patients was obtained from test requisition forms or by follow up with ordering health care providers. Mutations in each gene were combined into four histological subtypes (triple negative; HER2 positive; ER-positive,HER2-positive; and ER-positive,HER2 negative). Associations for each subtype were estimated by case-control analyses comparing the frequencies of pathogenic mutations in each subtype with frequencies from non-TCGA controls from the Exome Aggregation Consortium (ExAC) database. In addition, case-case analyses were conducted to assess enrichment of gene mutations in specific breast cancer subtypes. Among the observed associations between genes and breast cancer subtypes, mutations in *CHEK2* and *ATM* were highly enriched in luminal breast cancers and *BARD1* was specifically associated with TN breast cancer. Refining the spectrum of pathological correlates with mutations in hereditary breast cancer genes will aid gene specific cancer risk management, and may accelerate the development of novel gene-specific therapeutic interventions.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-09-04

Title: Assessment of breast cancer risk among atomic-bomb survivors based on *ATM* polymorphisms, radiation dose, and age at exposure

Hayashi T, Yoshida K, Ohishi W, Kyoizumi S, Kusunoki Y and Nakachi K. Radiation Effects Research Foundation, Hiroshima, Japan.

Body: RERF's epidemiological study on atomic bomb survivors has found increased incidence of several types of cancers with increased radiation dose. A great deal of inter-individual variation in susceptibility to radiation-associated breast cancer could not be explained solely by radiation dose, suggesting the possibility of partial involvement of DNA repair capacity. Of 11 known single nucleotide polymorphisms (SNPs) of the DNA repair gene *ATM*, we selected a particular SNP (located in the 5' untranslated region and therefore thought to be associated with *ATM* gene expression) and examined the association with radiation-associated breast cancer in the context of an RERF A-bomb survivor cohort study. A follow-up survey of 3,040 female members of the RERF Immunogenome Study Cohort during 1981-2009 identified a total of 108 breast cancer cases. *ATM* genotypes (*ATM-G/G*, *-G/A*, and *-A/A*) were determined by the TaqMan assay method for all cohort members. Relative risks (RRs) of breast cancer were estimated for radiation dose, the *ATM* genotypes, and the combined categories of dose levels and genotypes. As a result, RR of breast cancer significantly increased with increased radiation dose (RR = 1.46/Gy, 95% CI: 1.19-1.80). A-bomb survivors with *ATM-G/G* homozygote revealed a significantly-increased RR of breast cancer (RR = 1.74, 95%CI: 1.18-2.56), using as a reference the risk of those with *ATM-A/A* homozygotes combined with *ATM-G/A* heterozygotes. When dividing the subjects into two groups by age at exposure (under 20 years of age and over 20), RR of breast cancer significantly increased with radiation dose in the group aged less than 20 years at exposure (RR = 1.65/Gy, 95% CI: 1.29-2.11), but no significant increase in RR by genotype was observed. In the group aged over 20 years at exposure, there was no significant increase in RR of breast cancer by dose, but RR of breast cancer significantly increased among *ATM-G/G* homozygotes, using as a reference the risk of *ATM-A/A* homozygotes combined with *ATM-G/A* heterozygotes (RR = 2.28, 95%CI: 1.28-4.06). In all analyses, no interaction between RR of breast cancer and combinations of radiation dose and genotype was detected. These findings suggested that the *ATM* genotypes and radiation dose might affect the risk of breast cancer among A-bomb survivors and that the significance of such impact might differ by age at exposure.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-09-05

Title: A model with polygenic risk score and mammographic density predicts interval cancers

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University of California, San Francisco; New Mexico State University; University of Texas MD Anderson Cancer Center; San Francisco Coordinating Center and San Francisco Veterans Affairs Medical Center.

Body: Introduction:

Interval breast cancers present with clinical symptoms following a normal screening mammogram. They are associated with unfavorable biological features and with dense breasts. Models predictive of aggressive phenotypes may facilitate tailored screening for women at elevated risk of interval cancers. Polygenic risk scores (PRS) represent the cumulative effects of multiple single nucleotide polymorphisms (SNPs) and can be used to risk-stratify women. In prior reports, PRS is preferentially associated with screen-detected rather than interval cancers. We investigated methods to refine the PRS to preferentially predict interval cancers, and tested the performance of the PRS in joint models with mammographic breast density (MBD).

Methods:

We used data from 1058 breast cancer cases from The Cancer Genome Atlas (TCGA) as the discovery set for our PRS. We selected 107 SNPs from genomewide association studies of breast cancer risk for testing against tumor status at last follow-up in TCGA. Presence of tumor indicated recurrence, progression, or positive margins after resection. Women with tumor present at <100 days of follow-up were excluded. Suggestive associations ($p < 0.2$) were used to construct a PRS, calculated as the sum across all SNPs of the per-allele log-odds ratio multiplied by the number of risk alleles for each SNP. We tested the performance of the PRS in a nested case-control dataset with 471 cases (102 interval cancers, 369 screen detected) and 496 controls from the California Pacific Medical Center Research Institute cohort. Logistic regression was used to evaluate the association between PRS, MBD and interval cancers. Area under the receiver operating characteristic (AUROC) curve was used to measure discrimination.

Results:

Of 107 SNPs, 23 had suggestive associations with presence of tumor at last follow-up in TCGA. The 23-SNP PRS discriminated between women with interval cancers and controls, with AUROC 0.57 (95% CI 0.51-0.63). With the inclusion of MBD in the model, the AUROC was 0.68 (95% CI 0.62-0.74). Women in the highest PRS quintile had an unadjusted 2.07-fold odds (95% CI 1.05-4.07) of developing interval cancers compared with women in the lowest quintile; adjustment for MBD did not change the point estimate. The PRS also discriminated between women with interval and screen-detected cancers, although the findings did not reach statistical significance (AUROC 0.55, 95% CI 0.48-0.61). With the inclusion of MBD in the model, the AUROC was 0.63 (95% CI 0.57-0.69).

Discussion:

A PRS associated with presence of tumor at last follow-up was independently predictive of interval cancers relative to controls. Models with PRS and MBD discriminated between interval and screen-detected cancers, although MBD provided most of the predictive power. Our findings are limited by the size and low number of recurrences in TCGA. It is possible that tumor status largely reflects treatment received, and may only partially represent the biological pathways of interval cancers. Our results suggest that SNPs may potentially identify women at risk for developing interval breast cancer, although further validation is required.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-09-06

Title: Ovarian reserve and response to controlled ovarian hyperstimulation (COH) in breast cancer women with and without BRCA mutation

Mailliez A, Keller L, Menu-Hespel S, Plouvier P, D'Orazio E, Basson L, Pigny P, Bonnetterre J and Decanter C. Centre Oscar Lambret, Lille, France; Hôpital Jeanne de Flandre, Lille, France and Centre de Biologie Pathologie - CHRU, Lille, France.

Body: Background: 6300 new cases of breast cancer arise in young women under 40 each year in France. Some of them are BRCA 1 or 2 mutation carriers. Most of them receive a potentially gonadotoxic chemotherapy while they have not yet completed their family. Since 2011, a systematic proposal of ovarian reserve follow-up and fertility preservation by oocyte freezing is provided to each young early breast cancer (BC) patients (pts) of our program (NCT 01614704). Preliminary results were presented at the SABCS in 2013. We now investigate the impact of BRCA mutation on the ovarian reserve and the ovarian response to stimulation.

Methods: 115 young BC pts were systematically referred to a reproductive medicine centre before starting chemotherapy. Inclusion criteria were age 18 to 38, histologically confirmed invasive breast carcinoma, absence of metastases. According to their personal and familial history, genetic counselling was performed and if the patient met the criteria and agreed, BRCA genes were analysed. Pts in an adjuvant setting and who were asking for fertility preservation underwent COH during the interval between complete surgery and start of adjuvant chemotherapy. Ovarian stimulation protocol consisted in a conventional antagonist protocol with recombinant FSH starting on day 2 of the menstrual cycles. The GnRh antagonist was started on day of the COH and the final oocyte maturation was achieved by an injection of triptorelin 0.2 mg when at least 3 follicles reached 18 mm of diameter. All pts gave their informed consent for COH, egg/embryo freezing and follow-up.

Results: 115 pts achieved pre-treatment AMH and AFC assessment. 60 (52,1%) were eligible for COH in order to cryopreserve egg or embryos. BRCA analysis was performed in 83 pts. 23 did not meet the criteria or refused. 9 analyses are still in process. 17 (20,4%) pts were positive for BRCA mutation (BRCA1: 13; BRCA2: 4) and 66 were not. In the mutation carriers group (n=17), median age was 32 years (Range 25-37). Median initial AMH levels and AFC were 23 pmol/l (5.1–223) and 20 (6-100), respectively. Eight pts underwent COH. Median duration of stimulation was 9,5 days (8-13) with a median cumulative dose of gonadotropins of 2875 UI (1200-5450). The median number of vitrified oocytes was 5,5 (0-15). Two patients chose frozen embryo preservation (1 and 2 eggs respectively). In the non-carriers group (n=66), median age was 31 years (24-37). Median initial AMH levels and AFC were 23.4 pmol/l (0.8-136) and 24 (1-68). 27 pts underwent COH. Median duration of stimulation was 10 days (7-14). Dose of gonadotropins was 2700 UI (1365-5600). The median number of vitrified oocytes was 6 (0-18). The 3 patients chose eggs preservation (0.0 and 3 eggs respectively). There was no significant difference in the two groups.

Discussion/ Conclusion: Few studies stated that BRCA1 mutation may be associated with reduced ovarian reserve in healthy BRCA mutation carriers. Meiorow and al concluded that both healthy and BC BRCA mutation carriers demonstrated normal ovarian response in vitro fertilization cycles. Our results show that ovarian reserve of BRCA 1/2 mutations BC carriers do not differ from that of non-carriers. Response to COH seems similar in both groups too.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-09-07

Title: Impact of germinalcenter-associated nuclear protein polymorphisms on breast cancer risk and prognosis in a Japanese population

Kotani H, Ito H, Kuwahara K, Kuzushima K, Iwata H, Tsunoda N, Nagino M, Tanaka H and Matsuo K. Aichi Cancer Ctr. Hosp., Nagoya, Aichi, Japan; Epidemiol. & Prev., Aichi Cancer Ctr. Res. Inst., Nagoya, Aichi, Japan; Immunol., Aichi Cancer Ctr. Res. Inst., Nagoya, Aichi, Japan; Mol. Med., Aichi Cancer Ctr. Res. Inst., Nagoya, Aichi, Japan; Nagoya University, Nagoya, Aichi, Japan and Niigata Univ. Grad. Sch. Med. Dent. Sci., Niigata, Japan.

Body: Background: Germinal center-associated nuclear protein (GANP) is a phosphoprotein which is involved in mRNA export and the regulation of DNA recombination. We have previously demonstrated that deficiency of *GANP* led to spontaneous development of mammary gland tumors in a mouse model. In addition, we found that decreased *GANP* expression in human breast cancer tissue was an independent prognostic factor. Here, we conducted a case-control study and a retrospective cohort study to investigate whether single nucleotide polymorphisms (SNPs) of *GANP* are associated with sporadic breast cancer risk and prognosis in a Japanese population.

Subjects and Methods: Six hundred-ninety-four breast cancer cases and 1,376 age- and menopausal status-matched controls were selected within the framework of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center. Cases and controls were genotyped using an Infinium iSelect custom array (iCOGS, Illumina Inc., San Diego, CA, USA). We assessed 13 SNPs at the *GANP* locus, 2 SNPs (rs2839178 and rs11702450) were selected for further analysis by considering linkage disequilibrium. Conditional logistic regression methods were used to estimate odds ratios (ORs) and 95% confidence intervals. In addition, the survival impact of the two SNPs was retrospectively analyzed using the 694 breast cancer cases. To evaluate the effect of SNPs on overall survival (OS) and disease-free survival (DFS), multivariate Cox proportional hazards modeling was applied.

Results: Compared to the AA genotype of rs2839178, the GG genotype showed statistically significant associations with breast cancer risk (OR: 0.48, 95%CI:0.30–0.76, $P = 0.002$). In prognostic analysis, compared to those with the genotype AA at rs2839178, patients with AG or GG showed longer DFS (HR: 0.71, 95%CI: 0.49–1.04 and 0.42, 0.13–1.42, respectively, P for trend = 0.04). The GG genotype of rs2839178 also showed a positive tendency for longer OS although it was not statistically significant (HR: 0.69, 95%CI: 0.44–1.08, $P = 0.11$). We did not find that rs11702450 was associated with either breast cancer risk or prognosis.

Conclusion: This is the first study to investigate the association between *GANP* SNPs and breast cancer risk and prognosis. The direction of association with DFS was consistent with that of susceptibility. These results demonstrate that *GANP* SNPs presumably prevent the occurrence and malignant advancement of sporadic breast cancers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-09-08

Title: Molecular evaluation of Peruvian patients with hereditary breast cancer reveals a novel germline mutation in *BRCA1*

Ponce J, Vigil C, Araujo JM M, Castañeda C, Calderon G, Buleje JL L, Acosta O, Danos P, Huaman F, Guevara-Fujita ML L, Aguilar A, Pinto JA A, Gomez HL L and Fujita R. Breast Unit, Oncosalud-AUNA, Lima, Peru; Unit of Basic and Translational Research, Oncosalud-AUNA, Lima, Peru and Genetics and Molecular Biology Center, Universidad San Martin de Porres, Lima, Peru.

Body: Background

Breast cancer is the leading cancer in women worldwide, while in Peru is the second most frequent cancer with a high incidence of triple negative breast cancers (21%). There is no previous information about *BRCA1/BRCA2* mutations in Peruvian high-risk breast cancer patients. Prior studies from International diagnostic laboratories only presented results of our population as a pooled Hispanic data. Our aim was to characterize mutations in *BRCA1/BRCA2* genes in Peruvian patients with breast cancer with hereditary patterns.

Methods

We evaluated mutations in *BRCA1/BRCA2* genes by Sanger sequencing and large genomic rearrangements by multiplex ligation-dependent probe amplification (MLPA) in 18 families with hereditary breast cancer criteria identified at the Breast Unit of Oncosalud-AUNA (Lima-Peru). Molecular analysis was done in the facilities of Genetics and Molecular Biology Center at the San Martin de Porres University (Lima-Peru).

Results

Sequencing identified 4 pathogenic mutations in 4/18 families, three previously detected (*BRCA1*: c.302-1G>C y c.815_824dup10; *BRCA2*: c.5946delT) and a novel germline mutation in exon 15 of *BRCA1* (c.4647_4648dupAA, ClinVar SCV000256598.1) producing a frameshift variant. MLPA revealed 2 amplifications in exon 7 (duplication and triplication) in *BRCA1* in unrelated patients with potential pathogenic effects, one of this co-existed with the *BRCA2*: c.5946delT mutation. In addition, three variants of uncertain significance were found (c.140G>T, in exon 5 of *BRCA1* and c.464G>A and c.938C>T in exon 5 and 10 of *BRCA2*, respectively).

Conclusions

After a comprehensive evaluation we found an alteration rate of 27.8% (5/18) in *BRCA1/BRCA2* in families with criteria for hereditary breast cancer. We reported *BRCA1* c.4647_4648dupAA as a novel mutation. Further studies including a larger sample size of Peruvian patients should evaluate the prevalence or founder effect of this mutation in our population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-09-09

Title: Functional *IGF1R* variant predicts preeclampsia protection from invasive breast cancer: Novel California teachers study findings

Powell M, Von Behren J, Neuhausen S, Reynolds P and Benz C. Zero Breast Cancer, San Rafael, CA; Cancer Prevention Institute of California, Berkeley, CA; Buck Institute for Research on Aging, Novato, CA and Beckman Research Institute of City of Hope, Duarte, CA.

Body: Many studies have reported lower breast cancer risk in women who develop hypertension in pregnancy with a meta-analysis reporting hazard ratios of 0.86 for preeclampsia and 0.83 for gestational hypertension. Our prior work in the Marin Women's Study (MWS) demonstrated both a lower breast density and a lower risk of breast cancer in women with pregnancy-induced hypertension (PIH) if they possess the TT genotype of *IGF1R* SNP rs2016347.

Breast cancer in MWS women with PIH by IGF1R genotype

rs2016347 genotype	# with genotype	# breast cancer cases	% breast cancer cases
GG	91	8	8.79%
GT	195	14	7.18%
TT	88	0	0.00%

Fisher's exact = 0.008

The current study was designed to validate and expand upon these findings in the larger California Teachers Study (CTS) which consists of >130,000 female educators. From original participants a case-control study was established in 2012 consisting of all non-Hispanic white women with DNA samples that became cases since entry into the study (N = 2030) and controls without invasive or in situ breast cancer (N = 1552). The current study nests within this case control study. All participants with a self-reported history of preeclampsia were selected (81 cases/56 controls). *IGF1R* SNP rs2016347 was assessed by Taqman assay.

Results: Women with the TT genotype had an odds ratio (OR) of 0.38 when compared to the GG genotype after adjusting for potential confounders. Stratification by HR+/HR- cases and by age of first birth (AFB) resulted in statistically significant adjusted OR's of 0.26 for HR+ positive cases and 0.15 for women with AFB <30. Both showed significant trend effect for number of T alleles as shown below:

Preeclampsia and breast cancer in CTS

rs2016347 genotype	All cases (N=137)	HR+ cases (N=118)	AFB <30 (N=106)
TT vs GG	0.38 (0.13, 1.14)	0.26 (0.07, 0.89)*	0.15 (0.04, 0.56)*
GT vs GG	0.53 (0.19, 1.46)	0.57 (0.19, 1.74)	0.34 (0.12, 1.12)
Trend analysis	p = 0.09	p = 0.03*	p = 0.005*

* p < .05

Overall in the CTS, the adjusted hazard ratio for women with vs without preeclampsia was 0.94 (0.81, 1.08).

Conclusions: These results suggest significant breast cancer protection in women with preeclampsia that possess the TT genotype, specifically in those women with AFB <30, and for the development of HR+ breast cancer. The overall OR for all

women with the TT genotype was low at 0.38 but did not reach statistical significance. This analysis in a second cohort again demonstrates a lower risk of breast cancer in women with a hypertensive disorder of pregnancy possessing the same *IGF1R* variant.

Recent studies have associated the rs2016347 T allele with lower normal tissue expression of *IGF1R* mRNA, better survival in HR+ breast cancer, and improved pathological response to neoadjuvant chemotherapy. The protective T allele creates a new microRNA (miR-432) binding site within the *IGF1R* 3'UTR, offering a potential functional explanation for reduced mammary gland expression of this cancer-associated growth factor. This may interact with alterations of growth and metabolic factors characteristic of preeclampsia to imprint the immature gland with a lasting protective effect from later life breast tumorigenesis. If mechanistically substantiated, these findings could lead to a novel breast cancer prevention strategy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-10-01

Title: Adherence to healthy lifestyle behaviors in a predominantly Hispanic population of women undergoing screening mammography

Ali-Shaw T, Ueng WA A, Trivedi MS S, Yi H, David RR R, Vanegas A, Vargas JM M, Sandoval R, Wood J, Kukafka R and Crew KD D. Columbia University Medical Center and Teachers College, Columbia University.

Body: Background: The American Cancer Society (ACS) established 2012 guidelines focusing on healthy lifestyle behaviors for the prevention of cancer and other chronic diseases. In our study, we compared adherence to the ACS guidelines for cancer prevention across a spectrum of breast cancer risk in a predominantly Hispanic population of women undergoing screening mammography.

Methods: We conducted a cross-sectional study in Washington Heights, NY among non-smoking women with a body mass index (BMI) ≥ 18.5 kg/m² undergoing routine screening mammography. Participants completed a self-administered questionnaire on demographics, breast cancer risk factors, and lifestyle factors, including adherence to ACS guidelines for physical activity (≥ 2.5 hours/week of moderate physical activity), consumption of fruits and vegetables (≥ 5 servings/day), alcohol intake (≤ 1 serving/day), and self-reported height and weight (BMI of 18.5-24.9 kg/m²). We calculated a composite ACS adherence score, where each of the four main components were scored 0 for non-adherence, 1 for partial adherence, and 2 for complete adherence (range, 0-8), with scores of 6 to 8 representing optimal adherence. High breast cancer risk status was based upon a $\geq 1.67\%$ 5-year risk of invasive breast cancer according to the Gail model or meeting family history criteria for *BRCA* genetic testing. Logistic regression models were used to examine the association between demographics, breast cancer risk status, and health behaviors.

Results: From Nov 2014-Dec 2015, 18,681 presented for screening, 2846 (15%) were approached for enrollment, 2345 consented, and 1977 were evaluable for this analysis. The mean age was 58.8 years (SD 10.9), over three-quarters identified as Hispanic, nearly 40% had not graduated high school, 24% met high-risk criteria for breast cancer, and only 8.3% had optimal adherence to ACS guidelines for lifestyle behaviors. In multivariable analysis, older women, racial/ethnic minorities, and those with lower educational levels were less likely to adhere to ACS guidelines. After controlling for known confounders, breast cancer risk status was not associated with adherence to lifestyle factors.

Conclusion: Our findings indicated that there is a significant difference in adherence to ACS guidelines for cancer prevention based upon age, race/ethnicity, and educational level. Our study has identified a population of women who may be targeted for lifestyle modification and screening mammography may provide a teachable moment to discuss strategies for preventing breast cancer and other chronic diseases.

Multivariable analysis of optimal adherence to ACS guidelines

Covariates	Odds Ratio	95% Confidence Interval	p-value
Age at enrollment	0.97	(0.95, 0.99)	0.003
Race/Ethnicity			
Non-Hispanic White	1.00		
Non-Hispanic Black	0.39	(0.20, 0.73)	0.003
Non-Hispanic Other	0.46	(0.22, 0.98)	0.045
Hispanic	0.28	(0.17, 0.47)	<.001
Highest level of education			
Less than high school	0.16	(0.08, 0.30)	<.001
High school graduate or GED	0.14	(0.07, 0.29)	<.001
Vocational/technical school, some college	0.44	(0.24, 0.68)	<.001
Graduate/post-graduate/professional degree	1.00		

High breast cancer risk status	1.30	(0.80, 2.12)	0.286
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2016 San Antonio Breast Cancer Symposium

Publication Number: P5-10-02

Title: African Americans have more aggressive invasive lobular carcinoma subtypes and inferior early outcomes: SEER 2010-2013

Thomas A, Altekruze S, Avery TP P, Melin SA A, Howard-McNatt MM M and Schroeder MC C. University of Iowa, Iowa City, IA; National Cancer Institute, Bethesda, MD and Wake Forest University, Winston-Salem, NC.

Body: Introduction:

African Americans (AA) present more frequently with triple negative breast cancer (TN) and other aggressive breast cancer subtypes. Invasive lobular (ILC) breast cancer most commonly presents as estrogen receptor (ER)+, progesterone receptor (PR)+ and HER2-, though less frequently the more aggressive ER- or PR- luminal, TN or HER2+ subtypes occur. For women presenting with ILC 2010-2013, we report by race, differences in disease subtype, grade and stage at presentation and 2-year outcomes.

Methods:

We conducted a retrospective cohort analysis using Surveillance, Epidemiology and End Results Program. Women diagnosed with first primary malignant lobular breast cancer from 2010-2013 were included. Subtypes were categorized into four exclusive groups: ER+ and PR+ HER2-, ER+ or PR+ HER2-, TN and HER2+. Two-year survival was compared across race, and a multivariate cox model assessed overall survival.

Results:

ILC occurred less frequently in non-whites (Table 1). AA and other non-whites were younger at diagnosis than whites (p<0.001). AAs and other non-whites were less likely to have ER+ and PR+ HER2- disease (OR 0.85, p= 0.019 and OR 0.79, p=0.003 respectively). AAs had ILC of significantly higher grade and presented with more advanced stage disease than other race categories (p<0.001 for both). On multivariate analysis, survival was inferior for AA relative to whites (HR 1.32, p<0.010). Other non-whites had better survival than whites (HR 0.58, p=0.008). For AAs 2-year survival by disease subtype was: ER+ and PR+ HER2- (91.3%), ER+ or PR+ HER2- (90.5%), TN (59.5%), HER2+ (84.0%). For these subtypes, the proportion of women presenting with Stage IV ILC was 8.1%, 10.8%, 22.6% and 15.9% respectively.

Conclusion:

In this large, recent ILC cohort there were significant racial disparities in disease biology at presentation, with non-whites having more aggressive ILC subtypes, but only AAs having higher grade ILC. Short-term survival outcomes were inferior for AAs. Whether AAs presenting with advanced stage disease more frequently is due to biology or access to care is unknown. Further study of disease biology and healthcare delivery disparities could offer improved outcomes for AAs with ILC.

Table 1: ILC Characteristics by Race

	White	AA			Other non-white		
N	13,557	1,445			957		
ILC - % diagnoses per race category	9.6	7.2			5.8		
Rate of ILC (per 100,000 women of that race)	10.5	6.4			4.4		
Median Age	64	61			60		
	%	%	OR*	p	%	OR*	p
Subtype							
ER+ and PR+ HER2-	80.9	78.3	0.85	0.019	77.0	0.79	0.003
ER+ or PR+ HER2-	12.9	14.4	1.14	0.106	15.3	1.22	0.036
TN	1.5	2.1	1.44	0.064	2.1	1.40	0.158
HER2+	4.7	5.1	1.09	0.473	5.6	1.21	0.186
Stage							

I	40.5	36.4			39.0		<0.001
II	36.5	34.5			40.4		
III	17.0	19.9			14.9		
IV	6.0	9.2			5.6		
Grade (Differentiation)							
Well or Moderate	91.2	87.5			89.5		<0.001
Poor or Undifferentiated	8.8	12.5			10.5		
2-year survival	93.9%	90.0%			96.3%		

*compared to white (reference group)

Table 2: Multivariate Cox Model for 2-year Survival

	HR	p	95% CI	
Race				
White	ref			
AA	1.32	0.010	1.07	1.64
Other non-white	0.58	0.008	0.38	0.87
Subtype				
ER+ and PR+ HER2-	ref			
ER+ or PR+ HER2-	1.80	<0.001	1.51	2.16
TN	2.89	<0.001	2.07	4.03
HER2+	0.97	0.867	0.69	1.37
Stage				
I	ref			
II	1.91	<0.001	1.51	2.42
III	3.44	<0.001	2.71	4.37
IV	22.34	<0.001	17.78	28.07
Grade (Differentiation)				
Well or Moderate	ref			
Poor or Undifferentiated	1.40	0.001	1.14	1.71

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-10-03

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-10-04

Title: Spectrum of hereditary breast and ovarian cancer gene variants in an African American cohort

Shah PD D, Digiovanni L, Maxwell KN N, Bradbury AR R, Van Den Akker J, Kim S, Gil E, Simon MS S, Nathanson KL L and Domchek SM M. Bassett Center for BRCA, Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA; Color Genomics, Burlingame, CA and Karmanos Cancer Institute, Wayne State University, Detroit, MI.

Body: Background: Few reports describe the spectrum of mutations in breast and ovarian cancer predisposition genes found specifically in African Americans. **Methods:** 560 women who self-identified as African American (AA) from the University of Pennsylvania and Wayne State University were included in this IRB-approved, case-control study. Cases (n=218 with a personal history of breast and/or ovarian cancer) and controls (n=342 without breast or ovarian cancer) underwent germline genetic testing using the Color Genomics 19-gene breast and ovarian cancer risk panel. The subset of AA patients diagnosed with breast cancer ≤ 40 (n=185) was compared to an institutional cohort of white patients with breast cancer diagnosed ≤ 40 (n=189). **Results:** Of 218 AA cases, 70 had pathogenic or likely pathogenic (P/LP) mutations (*BRCA1*: n=36; *BRCA2*: n=24; *TP53*: n=3; *RAD51D*: n=2; *ATM*: n=2; *CHEK2*: n=2 and *MSH6*: n=1). Forty-two of 218 patients (19%) had at least one variant of uncertain significance (VUS). Of 342 AA controls, 5 women had P mutations in 5 distinct genes: *BRCA2*, *ATM*, *BRIP1*, *PALB2* and *PMS2*. 55 control patients (16%) had at least one VUS. Many of the 75 P/LP mutations (cases, 70 mutations; controls, 5 mutations) in the full AA cohort were unique variants. In the 135 patients who had *BRCA1/BRCA2* sequencing prior to testing under this protocol, the Color Genomics platform identified all 56 pathogenic mutations. Among AA patients diagnosed with breast cancer ≤ 40 (n=185), the incidence of *TP53* and *ATM* pathogenic mutations was similar to the white, early-onset breast cancer cohort (n=189): *TP53*, 1% in both cohorts; *ATM* = 1% in AA patients and 2% in whites. However, no patients in the AA, early-onset cohort had germline *CHEK2* mutations, compared to 4% of white, early-onset breast cancer patients (p=0.007). **Conclusions:** Taken together, the results of this study demonstrate the importance of considering germline mutation testing in the AA population. Examination of mutations and disease phenotypes within the AA population may facilitate understanding of the clinical risk associated with variants of uncertain significance. Further comparative data between the AA and white cohorts will be presented.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-10-05

Title: Time differences in breast cancer diagnosis among minorities in a large referral academic center

Acuna A, Gligich O, Khan H, Xue X, Lin J, Sparano J and Anampa J. Montefiore Medical Center/Albert Einstein College of Medicine.

Body: Background: Breast cancer (BC) is the most common malignancy and leading cause of cancer death in women. BC incidence is lower in Hispanic (H) (91.9/100,000) compared to non-Hispanic Whites (NHW -128.1/100,000) and Non-Hispanic Black (NHB - 124.3/100,000) population; however, mortality rate is higher in NHB (31/100,000) compared to NHW (21.9/100,000) and H (14.5/100,000). Diagnosis delay is a plausible factor that may explain differences in BC clinical outcomes among different race/ethnicity subgroups.

Objective: To compare time to diagnosis (TTD) by race/ethnicity in women with breast cancer diagnosed at Montefiore Medical Center from 2004 to 2012.

Methods: Patients with breast cancer and available race/ethnicity information diagnosed between 2004 and 2012 were categorized into 4 race/ethnicity groups: NHB, NHW, H or Asian. Dates of screening mammogram, diagnostic mammogram and biopsy were obtained. TTD was defined as the time difference between abnormal mammogram and biopsy dates.

Results: 919 patients had ethnicity information, 302 (32.8%) were H. TTD was longer in H compared to non-Hispanics (35 vs 31 days, $z=2.2$, $p=0.02$). Race and ethnicity information was available for 834 patients with a mean age of 62 years (SD:12.4). Of these, 252 (30.2%) were H and 387 (46.4%) were NHB. NHW had the shortest TTD (30 days), the highest frequency of Stage I (70%) and lowest frequency of high-nuclear grade (15.6%). NHB had a TTD of 31 days and higher frequency of triple negative disease (18.9%). TTD was significantly longer in H compared to NHW (35 vs 30 days, $z=2.3$, $p=0.02$), and there was a non-significant longer TTD when comparing H versus NHB (35 vs 31 days, $z=1.9$, $p=0.0574$). TTD between NHB and NHW was not different (31 vs 30 days, $z=1.4$, $p=0.14$).

Conclusions: The longer TTD in H vs Non-Hispanics was driven by the TTD in NHW. NHW had shorter TTD and more favorable pathological features which could lead to lower mortality rate. There was no difference in TTD between NHW and NHB but the latter had higher frequencies of triple negative disease. Correlation between TTD and mortality in our population will help to clarify the clinical effect of TTD differences among race/ethnicity subgroups.

	Total (n= 834)	Not Hispanic Black (n=387)	Not Hispanic White (n=180)	Hispanic (n=302)	Asian (n=15)
Age (SD)	62 (12.4)	62.9	65.2	61.6 (11.9)	53.2
Stage*					
I	613 (65.7)	239 (61.6)	126 (70)	202 (67.3)	10 (66.7)
II	242 (25.9)	121 (31.3)	36 (20)	71 (23.7)	3 (20)
III	56 (6)	19 (4.9)	12 (6.7)	20 (6.7)	3 (15.3)
IV	21 (2.3)	8 (2.1)	6 (3.3)	7 (2.3)	1 (1)
Histology*					
IDC	697 (75.1)	292 (75.5)	121 (67.2)	224 (74.7)	13 (86.7)
ILC	81 (8.7)	34 (8.8)	23 (12.8)	21 (7)	2 (13.3)
Mixed	140 (15.1)	57 (14.7)	33 (18.3)	49 (16.3)	0 (0)
Grade*					
High	253 (28.4)	138 (35.7)	28 (15.6)	73 (24.3)	4 (26.7)
Moderate	426 (48)	155 (40.1)	86 (47.8)	151 (50.3)	9 (60)

Low	210 (23.6)	83 (21.5)	49 (27.2)	62 (20.7)	1 (6.7)
Receptor status*					
ER positive	762 (81.8)	286 (73.9)	162 (90)	258 (86)	12 (80)
PR positive	632 (67.8)	229 (59.2)	137 (76.1)	219 (73)	11 (73.3)
HER-2 positive	143 (15.4)	70 (18.1)	14 (7.8)	49 (16.3)	5 (33.3)
Triple negative	115 (12.3)	73 (18.9)	14 (7.8)	21 (7)	1 (6.7)
Times					
Time to diagnosis	33 (20-52)	31 (19-52)	30 (19-44.5)	35 (21-58.5)	42 (21-72)
Screening to Diagnostic	22 (14-36)	22 (13-36)	21 (13-32)	23 (14-14)	26 (17-37)
Diagnostic to Biopsy	7 (0-13)	7 (0-13)	6 (0-14)	7 (1-13)	5 (0-9)

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-10-06

Title: Race associated disparities in an urban population of adolescent and young adult (AYA) women with breast cancer

Chitalia AA A, Aqbal D, Yin R, Cai L, Isaacs CJ J and Lynce FC C. Lombardi Comprehensive Cancer Center, Medstar Georgetown University Hospital, Washington, DC; Georgetown University, Washington, DC and Georgetown University School of Medicine, Washington, DC.

Body: Introduction: Although only about 5-7% of breast cancers occur in women under 40 years of age, multiple studies have shown that these cancers are more aggressive. In addition issues related to genetic testing and fertility preservation are of particular importance for AYA patients with breast cancer. This retrospective analysis aims to describe various biologic and treatment related factors of an urban AYA population with breast cancer and the differences amongst these factors stratified by African American (AA) versus non-AA race. We will look at factors including stage at diagnosis, hormone receptor status, prevalence of *BRCA1/2* mutations, time from diagnosis to treatment, enrollment in clinical trials, and fertility preservation.

Methods: A retrospective study of a population of AYA women with breast cancer seen at two hospitals in Washington D.C. from 2006 to 2015 was performed. Data was collected on age, *BRCA1/2* test results, stage at diagnosis, hormone receptor status, time from diagnosis to treatment, enrollment in clinical trial, fertility preservation, and pregnant or breastfeeding status at time of diagnosis. Fisher exact test was used to test the association between two categorical variables. Wilcoxon rank sum test was used to compare time to treatment and stage at presentation between AA and non-AA patients.

Results: A total of 161 AYA patients were evaluated. 54 were identified as AA and 107 as non-AA (88 Caucasians, 13 Asian, 6 Hispanics). Median age was 32 years (20-39) overall; 32 years (23-39) for AA and 33 years (20-39) for non-AA. While the rate of genetic testing was high, significantly fewer AA AYA underwent testing compared to non-AA (74% versus 87% respectively, (p=0.050)) and 10% of AA versus 22% of non-AA were found to have a *BRCA1/2* deleterious mutation (p=NS). Clinical trial participation was lower for AA compared to non-AA (57% vs. 76%, p=NS) for those where clinical trials were discussed/offered. Fertility preservation was pursued by 10% of AA vs. 35% of non-AA (p=0.001). Of note, 61% of AA and 34% of non-AA had children at the time of diagnosis (p=0.001). While AA (31%) presented more often with advanced stages of cancer compared to non-AA (19%) this was not statistically significant. Triple negativity was similar in AA AYA (25%) vs. non-AA (22%). The median time to treatment initiation did not vary between AA and non-AA (37 days vs. 36 days, p=NS).

Conclusion: In this retrospective study of an urban population of AYA women with breast cancer, there were no delays in treatment initiation or significant differences between AA and non-AA. When compared to non-AA, AA had a statistically significant lower rate of genetic testing and fertility preservation, although the latest could have been affected by a higher number of nulliparous in non-AA. . Encouragingly, the clinical trial participation of AYA who had previously discussed clinical trials with their physician was very high.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-10-08

Title: Estrogen receptor quantitative measures and differences in breast cancer survival

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Body: Introduction: In New Mexico, Hispanic women have a 1.7-fold increased risk of breast cancer-specific death compared to non-Hispanic white women. In previous studies, race/ethnic minority women have had larger survival disparities in estrogen receptor positive (ER+) than ER- disease, suggesting some aspect of ER may mediate survival outcomes. We thus conducted an extensive assessment of ER quantitative measures.

Objective: To determine whether ER percent positive and intensity differs by ethnicity, and to evaluate whether that potential difference might account for a proportion of survival disparities.

Methods: We conducted a population-based case-cohort study of first invasive breast cancer diagnosed in white females from 1997-2009 in six NM counties, identified through Surveillance Epidemiology End Results (SEER). We selected 15% of breast cancer cases and all breast cancer deaths through 2012. After IRB approval, pathology reports and tissue microarrays served as sources of ER, PR, and Her2 information. Tumors were classified according to modified intrinsic subtypes based on immunohistochemistry. Data were analyzed using Cox proportional hazards models adapted for case-cohort with weighted estimates (cohort weighted by 6.67x), and estimated hazard ratios (HR) and 95% confidence intervals (CI) using the robust variance and $\alpha=.05$. The proportional hazards assumption was verified by Schoenfeld residuals. All analyses were adjusted for age. Interaction was assessed by inclusion of main effects and a product term (subtype* exposure).

Results: ER and intrinsic subtype information was available for 76% of the cohort (867/1143) and 70% of breast cancer deaths (689/991). Median follow up was 7.8 years. In analyses stratified by intrinsic subtype, Hispanic women experienced elevated mortality relative to non-Hispanic whites for luminal A (HR 1.9;95% CI 1.4-2.6), Luminal B (HR 2.9;95% CI 1.5-5.7), and TN tumors (HR 1.9;95% CI 1.0-3.6) but not Her2+ER- disease (HR 1.1;95% 0.3-3.4).

Overall ER Quantitative measures: Among ER+ women, breast cancer mortality decreased with increasing ER+ staining, measured by percent (p-trend=.004) or quartile (p-trend=.002). After adjustment for ER percent(ER%+), women with increased ER intensity (score \geq 2) had reduced mortality, relative to score=1 (HR 0.6;95% CI 0.4-.1.0). Results did not differ by Luminal A or B subtype (p interaction> .05).

Ethnicity-specific ER quantitative measures: ER%+ distribution did not differ by Hispanic ethnicity. However, among Hispanic women, interaction terms for ER%+ (p=.04) or quartile (p=.08) by subtype in relation to breast cancer survival suggest that Hispanic women with increasing ER staining have a reduced risk of mortality in Luminal A but not Luminal B tumors. Such differences were not evident among non-Hispanic white women. In multivariate models, inclusion of ER%+ and staining intensity did not alter Hispanic survival disparity overall, but mediated 8.6% in Luminal B.

Conclusion: After inclusion of ER%+, ER staining intensity is an independent risk factor for breast cancer survival. Differences in ER quantitative measures appear to account for only a small proportion of survival disparities. Survival gaps in ER+ breast cancer may be attributable to host or other tumor factors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-10-09

Title: Prospective cross-sectional-study on participation in mammography screening according to immigration background and education status

Kuehnle E, Oeztuerk T, Siggelkow W, Luebbe K, Moser A, Noeding S, John J, Noesselt T, Busch C, Arfsten M, Lemster S, Hillemans P, Doerk T and Park-Simon T-W. Hannover Medical School, Hanover, Lower-Saxony, Germany; Diakovere Henriettenstift, Breast Center, Hanover, Lower-Saxony, Germany; Klinikum Hanover Nordstadt, Cooperativ Breast Center KRH, Hannover, Lower-Saxony, Germany; Helios Klinikum Hildesheim, Breast Center, Hildesheim, Lower-Saxony, Germany; Kreiskrankenhaus Stadthagen, Breast Center Schaumburg, Stadthagen, Lower-Saxony, Germany and Sana Klinikum Hameln-Pyrmont, Breast Center, Hameln, Lower-Saxony, Germany.

Body: Introduction

Although the health of immigrants is an important issue in national health care policy there is a serious shortage of data in many countries. Most studies lack information on educational status which is a major limitation. In this prospective cross-sectional-study we analyzed the influence of immigration background and educational status on the participation in mammography screening programs in Lower-Saxony, Germany.

Material and methods

Data collection was conducted from 2012-2016 in six certified breast cancer centers using a personal questionnaire and data from the patients' medical records. Stratification into subgroups was carried out according to first and second generation immigrants and country of origin.

Results

1547/2129 primary breast cancer cases were analyzed. The percentage of patients with a history of immigration in our study cohort was 17.7%. The majority of them were citizens of EU27 Member states. First generation immigrants (n= 146), second generation immigrants (n=129), natives (n= 1272). No significant difference was seen in sex, age, tumor stage, histology, grading, Ki-67, Her2/neu-status, and hormone receptor status. A 100% participation rate in the mammography screening program was seen in patients with no school graduation. The lowest participation rate (85.5%) was seen in the group of native Germans with a college graduation and in first generation immigrants with a high school graduation (86.7%). Detailed statistical analysis will be presented on the poster.

Conclusion

No difference was seen between immigrants and native Germans with regard to tumor biology. In first-generation immigrants mammography screening was well accepted despite cultural and linguistic differences. Participation rate decreased with higher education level in all groups. High school graduates with immigrant background participated more frequently in breast cancer screening than native high school graduates. These findings mainly relate to immigrants from EU27 Member states rather than immigrants from non EU countries.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-10-10

Title: Predictors of breast density among Black and Hispanic women presenting for mammographic screening

Oppong BA A, Dash C, Li Y, Makambi K, Coleman T and Adams-Campbell L. MedStar Georgetown University Hospital, Washington, DC; Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC; Georgetown University, Washington, DC and Capital Breast Care Center, Washington, DC.

Body: Background: Increased breast density has been associated with elevated breast cancer risk and complicating mammographic interpretation. Although previous studies have investigated population variations in breast density, Black and Hispanic women are often underrepresented in these analyses. Moreover, it is unclear how breast density differs between these ethnicities. We report on the mammographic density distribution of Black and Hispanic women having breast cancer screening at the Capital Breast Care Center (CBCC) and analyze factors associated with high breast density.

Methods: Retrospective data from electronic medical records at a population-based mammography screening center were abstracted. From 2010 to 2014, data from women undergoing their first breast cancer screening were reviewed. Patient demographics including race, age at screening, education and menopausal status were abstracted in addition to body mass index (BMI) and Breast Imaging-Reporting and Data System (BI-RADS) density category: 1- "fatty", 2- "scattered fibroglandular densities", 3- "heterogeneously dense" and 4- "extremely dense". Logistic regression was used to investigate factors associated with breast density.

Results: Density categorization was recorded for 1747 women over the five-year period, with 855 (49%) Black and 892 (51%) Hispanic. Patient characteristics associated with high density (categories 3 and 4) were younger age, Hispanic ethnicity, nulliparity, premenopausal status, and BMI < 30 kg/m². On multivariate logistic regression, Hispanic ethnicity, premenopausal status, and BMI < 30 kg/m² were predictive of high mammographic density.

Conclusion: In a sample of women presenting for mammographic screening at CBCC, Hispanic women were more likely to have higher breast density compared to Black women. After controlling for ethnicity, postmenopausal and obese women were less likely to have dense breasts. Additional investigation is needed to further study the impact of obesity on breast density in underserved minority women.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-01

Title: Self-evaluation of duration of adjuvant chemotherapy side effects in breast cancer patients: A prospective study

Galizia D, Martinello R, Cagnazzo C, Foresto M, Gallizioli S, Longo V, Berchiolla P, Solinas G, Calori A, Volpone C, Parola G, Tealdi G, Ballari A and Montemurro F. Candiolo Cancer Institute-FPO (IRCCS), Candiolo, Italy; AOU Città della Salute e della Scienza Torino, Presidio Sant'Anna, Turin, Italy; University of Turin, Turin; Ospedale Maggiore della Carità, Novara, Italy; Ospedale Cardinal Massaia, Asti, Italy; Azienda Sanitaria Locale Verbanò, Cusio, Ossola, Verbania, Italy; Azienda ASO Santa Croce e Carle, Cuneo, Italy and AOU Città della Salute e della Scienza Torino, Presidio Molinette, Torino, Italy.

Body: Background: Collection and analysis of chemotherapy-related side-effects (CSE) is critical in the management of cancer patients (pts) both in experimental trials and in the clinical practice. Usually, most of the available conventional systems like the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) capture CSE severity but not their duration. Recently we observed that self-evaluation of CSE incidence and severity by a CTCAE v4.0-based questionnaire was feasible and potentially more informative than doctor reports in pts undergoing standard adjuvant chemotherapy (ACT) for operable breast cancer (Montemurro et al., JAMA Oncol 2; 445-452, 2016). Our questionnaire had also a section for reporting, for each of the considered CSE, day of onset, duration and whether it was still present at the time of the visit. Here we report the analysis of patient self-evaluation of CSE duration.

Methods: The study prospectively enrolled 604 pts receiving ACT for operable breast cancer between January 2011 and October 2013 at 11 sites in Italy. CTCAE v4.0 definitions of grade of severity for nausea, vomiting, constipation, anorexia, dysgeusia, diarrhea, fatigue, pain, paresthesia, and dyspnea were translated into Italian and rephrased. Questionnaires were administered after the first and third cycle of chemotherapy. At each time-point, information on CSE was extracted from the medical charts and compared to patient questionnaires.

Results: Overall 1177 questionnaires were collected, 596 after cycle 1 and 581 after cycle 3 of ACT. A median of 82% of the fields was completely filled-in. 594 and 573 pts-questionnaires had a corresponding MD-questionnaire. Comparison of CSE duration after cycle 1 of chemotherapy as self-assessed by pts *versus* that reported by doctors is summarized in the table

Item (available paired data)	Patients		Doctors		P**
	Incidence (%)	Mean duration* (SD)	Incidence 8%	Mean Duration* (SD)	
Nausea (538)	67	3.7 (3.6)	40	1.6 (2.6)	<0.01
Vomiting (571)	22	1.8 (1.7)	11	1.2 (1.8)	<0.01
Constipation (546)	49	2.7 (3.4)	12	1.0 (2.9)	<0.01
Anorexia (563)	53	3.8 (4.2)	7	1.0 (1.9)	<0.01
Dysgeusia (556)	50	5.0 (5.7)	8	1.0 (4.1)	<0.01
Diarrhea (568)	15	2.4 (2.8)	4	0.9 (1.8)	<0.01
Fatigue (533)	75	6.7 (5.2)	25	0.9 (2.5)	<0.01
Pain (518)	32	3.6 (4.4)	10	0.5 (2.4)	<0.01
Paresthesia (582)	23	2.9 (5.2)	3	5.2 (0.8)	<0.01
Dyspnea (574)	25	6.2 (5.6)	2	5.0 (1.8)	<0.01

*5% trimmed mean, ** Student T test for paired samples

. For all CSE, patient reported longer duration than doctors did. Comparisons of reports after cycle 3 were similar.

Conclusions: self-reporting of CSE duration is feasible in patients receiving ACT after breast cancer surgery by using a CTCAE-derived questionnaire. Because doctors tend to underestimate both incidence and duration of CSE, patient-reported outcomes should be incorporated into the clinical practice because of the potential to provide a better estimate of the total burden

of CSE.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-02

Title: Impact of pre-operative exercise and mind-body interventions on patient-reported outcomes in women with newly diagnosed breast cancer

Ligibel JA A, Giobbie-Hurder A, Dillion D, Shockro L, Campbell N, Rhei E, Troyan S, Dominici L, Golshan M, Chagpar A, Yung R, Freedman R, Tolaney S, Winer E, Frank E, McTiernan A and Irwin M. Dana-Farber Cancer Institute; Brigham and Women's Hospital; Yale University and Fred Hutchinson Cancer Research Center.

Body: Background: Breast cancer diagnosis has a number of adverse psychological effects. The Pre-Operative Health and Body (PreHAB) Study tested the impact of exercise and mind-body interventions upon mood, quality of life, and patient-reported outcomes in women with newly diagnosed breast cancer.

Methods: Women with newly diagnosed Stage I-III breast cancer were enrolled through Dana-Farber Cancer Institute and Yale University breast cancer clinics prior to surgery. Participants were randomized 1:1 to an aerobic and strength-training exercise intervention, comprised of twice-weekly meetings with an exercise trainer and home based aerobic exercise, or to a self-directed mind-body relaxation intervention, comprised of a book and CD focused on relaxation and visualization. Participants engaged in the interventions between enrollment and surgery. The EORTC QLQ C-30, Hospital Anxiety and Depression Scale, and Perceived Stress Scale were collected at enrollment and prior to surgery.

Results: 49 women were randomized (27 exercise and 22 control). Mean time between enrollment and surgery was 4.2 weeks. At baseline, patients reported moderate levels of anxiety, stress, insomnia, and lack of appetite, as well as diminished emotional and cognitive functioning (Table). Exercise participants significantly increased minutes of weekly exercise vs. mind-body participants (increase of 203 vs. 23 min/wk, $p < 0.0001$). Mind body participants engaged in the intervention on average 69% of days during the intervention period. Pre-post changes demonstrated that participation in the mind-body intervention led to improvements in emotional and cognitive functioning and a reduction in anxiety and stress, and participation in the exercise intervention led to improvements in global quality of life, insomnia, appetite, and stress (Table). Women in the mind-body group experienced a significantly greater improvement in cognitive functioning as compared to women in the exercise group.

Conclusions: Women with newly diagnosed breast cancer reported a number of physical and psychological symptoms in the pre-operative period. Exercise and mind-body interventions demonstrated promising benefits in improving functioning and reducing symptoms. More work is needed to develop pre-operative programs to help reduce the distress imparted by a cancer diagnosis in the critical time between diagnosis and surgery.

Table*

	Exercise			Mind Body			Between Groups
	Baseline	Change	p value	Baseline	Change	p value	p value
Emotional Functioning	68.6 (23.3)	4.7 (18.3)	0.29	66.3 (24.2)	10.0 (20.5)	0.05	0.64
Cognitive Functioning	79.5 (24.6)	-3.3 (24.1)	0.62	73.8 (22.1)	11.7 (15.4)	0.002	0.03
QOL	74.0 (15.3)	9.7 (15.9)	0.005	69.0 (21.1)	7.5 (18.5)	0.11	0.78
Insomnia	35.9 (32.6)	-16.7 (32.6)	0.03	34.9 (35.7)	-8.3 (21.3)	0.06	0.52
Lack of Appetite	17.9 (27.0)	-13.3 (27.2)	0.03	11.1 (19.2)	-5.0 (22.4)	0.53	0.29
Anxiety	8.3 (3.4)	-0.6 (2.9)	0.25	9.2 (2.5)	-1.6 (2.3)	0.006	0.35
Stress	14.7 (7.2)	-2.2 (4.9)	0.06	18.4 (5.5)	-3.1 (6.8)	0.06	0.77

*Results reported as means (SD). Positive scores on functional and QOL measures indicate improvements; negative scores on symptom measures indicate a decrease in symptoms.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-03

Title: Difference between 1st and 2nd generation serotonin receptor antagonists in triplet antiemetic therapy for highly emetogenic chemotherapy in breast cancer patients – according to recent multi-institutional double-blind randomized clinical research on the AC regimen

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Body: <Background>

The information presented herein extends our recent study of TTT (Trial for Triplet Antiemetic Therapy).

One of our main clinical questions has been whether a 2nd generation serotonin receptor antagonist (5-HT₃ra) would be superior to 1st generation 5-HT₃ra when administering triplet antiemetic therapy for the prevention of chemotherapy induced nausea & vomiting (CINV), since a prior Japanese trial demonstrated palonosetron to be superior to granisetron for controlling the delayed phase of CINV induced by highly emetogenic chemotherapy (HEC) and to doublet antiemetic therapy including dexamethasone for anthracycline and cyclophosphamide containing regimens (AC).

<Objectives>

In this study, we assessed the efficacies of 1st and 2nd generation 5-HT₃ra agents for use as triplet antiemetic therapy for AC, by monitoring CINV, focusing especially daily CR in the delayed phase.

<Material and method>

Between 2012 and 2015, 491 women with breast cancer receiving AC were recruited from 11 institutions in Japan, and randomly assigned to either single-dose palonosetron (0.75mg) or granisetron (40µg/kg) prior to chemotherapy on day 1, both with dexamethasone (9.9 mg intravenously) and aprepitant (125mg orally) on day 1 followed by additional doses (80mg orally) on days 2 and 3. Age, institution and habitual alcohol intake were used as stratification factors. The primary endpoint was a complete response (CR). Statistical analysis was done by Mantel-Haenszel Method. This trial was registered with UMIN000007882.

<Results>

All 491 patients were included in efficacy analyses (ITT): 246 patients in the palonosetron group and 245 in the granisetron group. We previously reported that the difference in CR during the delayed phase, i.e. 24 hours after the administration of AC, did not reach statistical significance (53.8% vs 58.5%) in MASCC 2016. However, daily CR in the palonosetron group was much higher than that in the granisetron group after 48 hours.

<Conclusions>

Palonosetron showed better efficacy in controlling CINV during the late period of the delayed phase, i.e. 48 hours after AC administration, than granisetron as triplet antiemetic therapy for AC.

<Considerations>

The pattern of CINV reportedly shows two peaks including an acute phase caused by serotonin and a delayed phase caused by substance P, though the pattern of CINV with triplet antiemetic therapy administration might be different if the suppression of each of these peaks were to be achieved more efficiently. CINV may not be divided into two phases in the future, or the borderline area between the acute and delayed phases may require revision. The analysis of the late period of the delayed phase was ad hoc in this trial. However, when conducting studies related to CINV, it might be worthwhile to revise the borderline area between the two phases of CINV to facilitate elucidating the mechanisms underlying this potentially debilitating side effect of chemotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-04

Title: Technology as a change agent for improving breast cancer quality care

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Body: Background: With rapid advances in research, clinicians often struggle to remain current with evolving care guidelines and to implement current national quality standards (NQS) relevant to breast cancer management. Adherence to NQS is driving reimbursement for cancer services, but clinical workflow processes and IT solutions are lacking to effectively document adherence. The Carevive Care Planning System™ (CPS), an evidence-based, patient assessment and care planning software, is designed to close gaps in quality cancer care by marrying clinical and patient-reported data with evidence-based algorithms to help centers improve and document their adherence rates to quality care standards.

Methods: This study enrolled 30 non-metastatic breast cancer patients presenting to an NCI-designated comprehensive cancer center for no greater than their second medical oncology visit, and compared provider adherence to quality metrics for these patients with 30 matched historical controls who were seen prior to the study intervention. All were planned for chemotherapy treatment. The two part study intervention included 1) Provider participation in certified continuing medical education (CME) on evidence-based assessment, decision-making, and management strategies for breast cancer and 2) Use of the Carevive CPS with intervention subjects, each of whom who completed a electronic survey assessing current symptoms and concerns prior to their visit, and then received a provider-approved care plan including tailored recommendations for symptom management and referrals. The primary aim was to compare provider adherence to select quality metrics between historical controls (pre-test) and post-intervention subjects.

Analysis/Results:

Patient enrollment began in July 2015 and an earlier report of control data showed improved provider knowledge post-CME and opportunities to improve adherence. Median age and distribution of race, ethnicity, breast cancer stage, and HER2/ER status was not statistically different between the groups. Provider adherence to quality standards from pre to post-test is shown below:

Quality Standard Metrics

Quality Standard	N	Pre	Post	Chi-square	p
Assessed emotional well being	60	20%	50%	6.19	0.045
Addressed emotional well being	21	33.3%	93.3%	8.51	0.004
Pain quantified by second visit	60	100%	100%	N/A	N/A
Pain plan documented	11	37.5	33.3%	<1	0.90
Opioid assessed post treatment	27	91.0%	100%	1.51	0.22
Opioid induced constipation assessed	27	18.2%	9.3%	<1	0.33

Conclusions: Provider adherence to quality metrics for emotional wellbeing increased from pre- to post- intervention, but did not for pain assessment and management. This was largely due to ceiling effect, but opportunities exist for continued improvement in pain management, at least in documentation. The Carevive CPS plus CME has the potential to allow institutions an patient-centered and user-friendly approach to both improve and document adherence to quality metrics.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-05

Title: Examining bone degeneration as a side effect of treatment in premenopausal breast cancer patients

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Body: Background: Although it is widely known that adjuvant treatments cause osteoporosis in postmenopausal women, the effect of treatment and the exact degree of bone loss in premenopausal women is not well understood. For this reason, this study examined the relationship between the changes of bone mineral density(BMD) and the types of treatment in premenopausal breast cancer patients.

Method: This retrospective study looks at preoperative and annual spine bone densitometry tests conducted on breast cancer patients during their observational period following initial treatment for their diagnosis. 461 premenopausal patients who received treatment from January 2006 to April 2007 were the study cohort because of the availability of bone mineral density (BMD) data (L-spine and femur T scores) for a two-year period. The data from the patients were separated into groups according to their treatment plans: observation, adjuvant chemotherapy, adjuvant chemotherapy followed by use of Tamoxifen, and GnRHa(Gonadotropin Releasing Hormone agonist) followed by Tamoxifen.

Results: Of the 461 patients, 21 received no treatment aside from surgery, 75 received only Tamoxifen, 34 only chemotherapy, 166 chemotherapy and Tamoxifen, and 165 Zoladex and Tamoxifen. The no treatment group demonstrates the standard annual change in BMD. At first year, the chemotherapy only group and tamoxifen after chemotherapy group showed significant bone loss in the first year ($p<0.005$, both). The patients who received tamoxifen alone or tamoxifen with goserelin showed decrease BMD but not significant. After 2 years, tamoxifen with goserelin group showed significant decreased BMD in both spine and femur ($p<0.001$, $p=0.001$, both). Chemotherapy group showed partial recovery from the first year bone loss. Conclusion: The patients who received chemotherapy or GnRHa as treatment showed the greatest degree of bone degeneration but at different periods. However, Tamoxifen used along with these treatments seemed to lessen the extent of bone degeneration. Significant bone loss from hormone treatment occurred two years after surgery as compared to one year for chemotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-06

Title: BEAUTY and the breast: Is adjuvant chemotherapy the right time for a beauty boost? Results of a randomised controlled trial

Saghatchian M, Lacas B, Deneuve J, Bouvet N, Mokdad-Adi M, Ghouadni A, Amiel P, Charles C, Boinon D, Delaloge S and Dauchy S. Institut Gustave Roussy, Villejuif, France.

Body: Background:

Beauty treatments (BT) commonly offered at the hospital meet a great success among patients (pts) as well as a favorable public image. However their precise modalities and benefits have not been assessed so far. Breast cancer (BC) pts are a group of special interest in this field.

Methods:

A total of 400 pts treated with adjuvant chemotherapy (CT) after surgery for primary BC were randomized to receive BT during the course of their CT or no BT (control).

Pts had to fill in 3 questionnaires (quest.) before initiation of CT (T0) and after their last CT (T1): EORTC QLQ C30, BR23 (BC specific quest.) and Body Image Scale (BIS).

Primary outcome was improvement in the Body Image scale of BR23 (BRBI) and in BIS. BRBI was analyzed in 2 categories (Poor BRBI < 75, and Good BRBI >= 75).

Statistical analyses were based on comparison of mean scores and adjusted on use of BT and score before CT. A qualitative assessment of pts' experience was performed at each cycle through an ad hoc quest.

Results:

Pts characteristics were similar in both groups: median age (50 years), T, N, and mastectomy rate (40%). A total of 1120 BT were administered.

Based on QLC-BR23 data, it was expected that 26% of BC pts would have a *Poor* BRBI. In our study, 50% of pts had a *Poor* BRBI at T0, correlated to younger age (<50), mastectomy and axillary lymph node dissection.

At T1, approximately 70% of pts had a *Poor* BRBI, similar in both groups (with and without BT) (p = 0.68). The majority of pts (86%) with a *Poor* BRBI before CT did not improve after CT. For 46% of pts with a *Good* BRBI prior to CT, BRBI turned *Poor* after CT.

Table 1 shows the results of the scores at T0 and T1 and their statistical comparison.

Outcome	Arm	N	Before CT , mean (SD)	After CT , mean (SD)	Change, mean (SD)	t-test
QLQ_C30 (scale 0-100) 0 = poor QoL, 100 = good QoL						
Global health status	BT	142	65.1 (19.6)	59.0 (18.1)	-6.0 (21.9)	0.13
	No BT	135	66.8 (19.2)	56.9 (18.1)	-9.9 (20.7)	
Role Functioning	BT	146	74.8 (25.0)	58.7 (30.3)	-16.1 (29.7)	0.48
	No BT	135	70.2 (27.2)	56.8 (31.3)	-13.5 (33.1)	
Social Functioning	BT	143	79.3 (24.3)	62.0 (30.5)	-17.2 (28.9)	0.14
	No BT	134	80.2 (25.0)	58.0 (29.8)	-22.3 (27.6)	
QLQ-BR23 (scale 0-100) 0 = poor QoL, 100 = good QoL						
Body image	BT	138	64.5 (32.3)	50.9 (31.0)	-13.5 (27.1)	0.88
	No BT	131	65.8 (28.6)	51.8 (31.3)	-14.0 (27.8)	
Sexual functioning	BT	134	27.7 (27.3)	20.4 (22.4)	-7.3 (22.0)	0.40

	No BT	125	26.1 (26.2)	21.3 (25.5)	-4.8 (26.1)	
BIS (scale 0-30) 0 = good QoL, 30 = poor QoL						
	BT	148	10.5 (7.8)	13.7 (7.7)	3.1 (6.9)	0.96
	No BT	146	9.9 (7.3)	13.1 (7.5)	3.2 (7.1)	

(SD: Standard Deviation)

Analyses of the qualitative assessment of pts' perception showed the perceived added-value and limits of BT at this stage of the BC care pathway.

Conclusion:

In our randomized study, 50% of women had a degraded body image before CT. CT induced an observed further degradation leading to very low scores, without any effect of BT. The qualitative assessment of pts' perceptions revealed the specific needs of pts in terms of BT at this stage of their pathway.

Our study highlights the underestimation of pts-perceived body-image at this critical phase of their disease and generates hypothesis for further designing and tailoring of BT as an impactful supportive tool for BC pts.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-07

Title: Pharmacokinetics of eflapegrastim in a phase 2 open-label dose-ranging study in breast cancer patients receiving TC regimen

Vacirca JL L, Papai Z, Horvath Z, Makharadze R, Reddy G, Song T, Koli P and Schwartzberg LS S. North Shore Hematology/Oncology Associates, East Setauket, NY; State Health Center, Budapest, Hungary; University of Debrecen, Oncology Clinic, Debrecen, Hungary; Cancer Center of Adjara Autonomous Republic, Batumi, Georgia; Spectrum Pharmaceuticals, Irvine, CA and West Cancer Center, Memphis, TN.

Body: Background: Eflapegrastim (SPI-2012/HM10460A) is a novel, long acting recombinant human granulocyte colony-stimulating factor (rhG-CSF). Eflapegrastim consists of an rhG-CSF conjugated to the recombinant *E coli* derived Fc fragment of IgG4 via a polyethylene glycol linker. Eflapegrastim is in clinical development for the treatment of chemotherapy induced neutropenia in cancer patients.

Methods: Pharmacokinetics (PK) of eflapegrastim was investigated in an open label, dose-ranging Phase 2 study in breast cancer patients receiving docetaxel + cyclophosphamide (TC) chemotherapy. The study consisted of 4 arms. Patients in Arms 1 through 3 received subcutaneous doses of 45, 135, or 270 µg /kg of eflapegrastim and patients in Arm 4 received 6 mg pegfilgrastim (Neulasta®) on Day 2 of each 21-day chemotherapy cycle. Serum samples were collected from a subset of eflapegrastim patients at pre-specified time-points and analyzed for eflapegrastim by a validated enzyme-linked immunosorption assay (ELISA). Pharmacokinetic analyses were conducted on serum concentration-time profiles after dosing in Cycle 1. The serum concentrations for samples collected in Cycle 3 were compared with the corresponding concentrations in Cycle 1. Pharmacokinetic analyses were not conducted for pegfilgrastim patients.

Results: The PK profile of eflapegrastim was investigated in 11 patients, including 3 patients in the 45 µg/kg treatment arm, 4 patients in the 135 µg/kg treatment arm, and 4 patients in the 270 µg/kg treatment arm. Following single eflapegrastim doses of 45, 135, or 270 µg/kg, peak serum concentrations increased in a dose proportional manner. The summary of pharmacokinetics of eflapegrastim is presented in the Table below.

Pharmacokinetic Parameters of Eflapegrastim in Patients Following Single Subcutaneous Doses in Cycle 1

Parameter	Eflapegrastim 45 µg/kg/	Eflapegrastim 135µg/kg	Eflapegrastim 270 µg/kg
C_{max} , ng/mL	N = 3; Mean = 7.00; SD = 6.08	N = 4; Mean = 247; SD = 276	N =3; Mean = 299; SD = 329
T_{max} , h ^a	N =2; Mean = 58.7; SD = 46.9 - 70.5	N = 4; Mean =9.00; SD = 8 - 48.1	N = 3; Mean = 24.00; SD = 24 - 24.1
AUC₀₋₃₁₂ , ng•hr/mL	N = 0; Mean = NC; SD = NC	N = 2; Mean = 16000; SD = 5850	N = 3; Mean = 22900; SD = 25100
t_{1/2} , h ^b	N = 0; Mean = NC; SD = NC	N = 2; Mean = 81.0; SD = 88.4	N = 1; Mean = 31.5; SD = NC

AUC = area under the concentration-time curve; C_{max} = maximum serum concentration; h = hour; NC = not calculated; SD = standard deviation; t_{1/2} = half-life; T_{max} = time to maximum serum concentration; a) Expressed as median and range; b) Expressed as harmonic mean and pseudo SD

The maximum serum concentrations of eflapegrastim in Cycle 3 increased with the dose of eflapegrastim. The serum concentrations of eflapegrastim in Cycle 3 were generally lower than those in Cycle 1, but the profile was similar to Cycle 1.

Conclusions: The C_{max} and AUC₍₀₋₃₁₂₎ of eflapegrastim increased in a dose proportional manner following subcutaneous administration. The half-life of eflapegrastim ranged from 31.5 to 81.0 hours, which is consistent with the half-life of other long-acting myeloid growth factors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-08

Title: Immunogenicity of eflapegrastim in a phase 2 open-label dose-ranging study of eflapegrastim in breast cancer patients receiving TC regimen

Vacirca JL L, Papai Z, Agajanian R, Horvath Z, Makharadze R, Ibrahim E, Koli P, Reddy G, Tedesco KL L, McGregor K and Schwartzberg LS S. North Shore Hematology/Oncology Associates, East Setauket, NY; State Health Center, Budapest, Hungary; The Oncology Institute of Hope and Innovation, Downey, CA; University of Debrecen, Oncology Clinic, Debrecen, Hungary; Cancer Center of Adjara Autonomous Republic, Batumi, Georgia; Beaver Medical Group, Highland, CA; Spectrum Pharmaceuticals, Irvine, CA; New York Oncology Hematology (US Oncology/McKesson Specialty Health), Albany, NY; Samaritan Hematology and Oncology Associates, Corvallis, OR and West Cancer Center, Memphis, TN.

Body: Background: Eflapegrastim (SPI-2012/HM10460A) is a novel, long-acting recombinant human granulocyte colony-stimulating factor (rhG-CSF). Eflapegrastim consists of an rhG-CSF conjugated to a recombinant E. coli derived Fc fragment of IgG4 via a polyethylene glycol linker. Eflapegrastim is in clinical development for the treatment of chemotherapy induced neutropenia in cancer patients.

Methods: Immunogenicity of eflapegrastim was investigated in an open label, dose-ranging Phase 2 study in breast cancer patients receiving docetaxel + cyclophosphamide (TC) chemotherapy. The study consisted of 4 arms. Patients in Arms 1 through 3 received subcutaneous doses of 45, 135, or 270 µg/kg eflapegrastim and Arm 4 received 6 mg pegfilgrastim (Neulasta®) on Day 2 of each 21-day chemotherapy cycle. Blood samples for immunogenicity analysis were collected before the start of each chemotherapy cycle (Day 1) and at the End-of-Study Visit. Samples were tested in a screening assay for Anti-Drug Antibodies (ADA) to eflapegrastim by a validated enzyme linked immunosorption assay (ELISA). Positive samples from the screening assay were further tested in a confirmatory assay for antibodies binding to eflapegrastim or G-CSF. Samples found positive in the confirmatory assay were further tested in a validated cell based neutralizing antibody assay.

Results: Serum samples from 143 patients in the study were tested for ADA to eflapegrastim and G-CSF. Preexisting antibodies binding to eflapegrastim or G-CSF were detected in 9 out of 143 (6.3%) patients. One out of the 27 patients (3.7%) in the Pegfilgrastim Arm who was negative prior to dosing was positive for ADA in the G-CSF confirmatory assay. Two out of 100 patients (2.0%) treated with eflapegrastim, who were negative prior to dosing, demonstrated treatment-induced formation of ADA in the G-CSF confirmatory assay. However, the responses in these patients were transient (ie, not consistently positive at all the sampling times) and the assay response values were low and only slightly above the plate-specific cut points. None of the patients tested were positive for G-CSF neutralizing antibodies. A formal assessment of the impact of serum ADA on the PK of eflapegrastim was not performed since PK was examined in only a limited number of patients and all of those patients were negative for ADA both at study initiation and post-dose.

Conclusion: No neutralizing antibodies against eflapegrastim or G-CSF were detected in patients administered eflapegrastim in this study.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-09

Title: Sustained efficacy of eflapegrastim in breast cancer patients in a phase 2, open-label, dose-ranging study

Vacirca JL L, Agajanian R, Papai Z, Horvath Z, Makharadze R, Ibrahim EN N, Choi MR, Song T, Tedesco KL L, McGregor K and Schwartzberg LS S. North Shore Hematology/Oncology, East Setauket, NY; The Oncology Institute of Hope and Innovation, Downey, CA; State Health Center, Budapest, Hungary; University of Debrecen, Oncology Clinic, Debrecen, Hungary; Cancer Center of Adjara Autonomous Republic, Batumi, Georgia; Beaver Medical Group, Highland, CA; Spectrum Pharmaceuticals, Irvine, CA; New York Oncology Hematology (US Oncology/McKesson Specialty Health), Albany, NY; Samaritan Hematology and Oncology Associates, Corvallis, OR and West Cancer Center, Memphis, TN.

Body: Background: Eflapegrastim is a distinct biologic that uses the innovative proprietary long-acting protein/peptide discovery technology (LAPSCOVERY™) and consists of a novel, modified recombinant human G-CSF conjugated to the Fc fragment of IgG4 via a polyethylene glycol linker. A Phase 2 study of 3 doses of eflapegrastim vs pegfilgrastim was conducted in breast cancer patients receiving docetaxel + cyclophosphamide (TC) chemotherapy.

Methods: This was an open-label, global, multicenter, dose-ranging study designed to compare the safety and efficacy of eflapegrastim relative to a fixed dose of pegfilgrastim as a concurrent active control. The study included 4 treatment arms: 3 dose levels of eflapegrastim (45 µg/kg, 135 µg/kg, and 270 µg/kg) vs pegfilgrastim (6 mg). The primary objective of the study was the Duration of Severe Neutropenia (DSN) during Cycle 1. The results for the primary objective, along with demographics and safety, were described in a previous presentation (SABCS 2015 P1-10-05). The secondary endpoints included DSN in Cycles 2-4, absolute neutrophil count (ANC) in Cycles 1-4, the overall incidences of febrile neutropenia (FN) and hospitalization rates.

Results: A total of 147 evaluable patients were enrolled. Patient and tumor characteristics were comparable across all 4 treatment arms. Median age was 59.0 years (range 32 to 77 years); most patients were <65 years (68%), Female (98%), and White (95%). The DSN for the 135 µg/kg and 270 µg/kg was non-inferior to pegfilgrastim during all cycles and the DSN for patients treated with 45 µg/kg was non-inferior during Cycles 2 and 3 (Table 1). The ANC was dose proportional across all 4 cycles. The incidence of FN and hospitalization rates was low in all arms and there were no significant differences between the Eflapegrastim and Pegfilgrastim Arms (Table 2).

Table 1. Duration of Severe Neutropenia in Cycles 2 to 4 of TC Chemotherapy by Treatment Arm

DSN (Days)	Eflapegrastim 45 µg/kg (N=39)	Eflapegrastim 135 µg/kg (N=36)	Eflapegrastim 270 µg/kg (N=36)	Pegfilgrastim 6 mg (N=36)
Cycle 2				
Difference with pegfilgrastim	0.38	0.04	-0.05	NA
Non-Inferiority p-value	0.001	<0.001	<0.001	NA
Cycle 3				
Difference with pegfilgrastim	0.31	0.02	0.01	NA
Non-Inferiority p-value	0.002	<0.001	<0.001	NA
Cycle 4				
Difference with pegfilgrastim	0.94	0.07	-0.02	NA
Non-Inferiority p-value	0.781	<0.001	<0.001	NA

DSN = Duration of Severe Neutropenia; NA = Not Applicable

Table 2. Incidence of Febrile Neutropenia and Hospitalizations

	Eflapegrastim 45 µg/kg (N=39)	Eflapegrastim 135 µg/kg (N=36)	Eflapegrastim 270 µg/kg (N=36)	Pegfilgrastim 6 mg (N=36)
Febrile Neutropenia				
Incidence (%)	3 (7.7%)	1 (2.8%)	1 (2.8%)	2 (5.6%)
Difference with Pegfilgrastim	2.1 %	-2.8%	-2.8%	NA
p-value	1.000	1.000	1.000	NA
Hospitalizations				
Incidence (%)	3 (7.7%)	3 (8.3%)	1 (2.8%)	5 (13.9%)
Difference with Pegfilgrastim	-6.2%	-5.6%	-11.1%	NA
p-value	0.469	0.710	0.199	NA

Conclusions: In breast cancer patients treated with TC, the non-inferiority of **DSN** of 135 µg/kg and 270 µg/kg eflapegrastim, compared to pegfilgrastim in Cycle 1, was sustained through Cycles 2-4 and the ANC profiles were comparable in Cycles 1-4. In addition, the overall incidence of FN and hospitalizations was comparable between the eflapegrastim arms and the pegfilgrastim arm.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-10

Title: Evaluation of the relationship between psychological distress and risk for breast cancer

Vennard K, Crocker A, Ogheneruona A, Cuke M and Wood M. University of Vermont Medical Center.

Body: Introduction: Women at increased risk for breast cancer (due to family history or having a pathogenic germline mutation in a cancer causing gene) have greater distress levels, which can impact quality of life and screening behavior. The goal of our study was to evaluate distress related to different risk factors for breast cancer and examine the stability of this distress over time. Methods: Women at increased risk for breast cancer who enrolled in the High-Risk Breast Program (HRBP) at the University of Vermont Medical Center were eligible for participation. Women with strong family history, a genetic mutation or atypia on biopsy (benign breast disease, BBD) who completed the Impact of Event Scale (IES) at least once were identified. The IES is a self-reported measure designed to measure subjective distress within the past seven days in relation to a stressor. The scale is based on a two-factor intrusion-avoidance model. Linear regression was used to examine change in distress over time and to compare distress levels between risk groups. Both risk measures and distress scores were examined using bivariate plots and normal probability plots. Pearson correlation coefficients and least squares regression coefficients were obtained along with 95% confidence intervals. In addition to treating the risk estimates as continuous measures, these estimates were grouped into low or average risk ($\leq 2\%$) over five years, moderate risk (2%-4%) over five years, and high risk ($\geq 5\%$) over five years. Using the ordinal classification into three risk groups, analysis of variance was conducted to detect group differences in average psychological distress using a 5% significance level.

Results: The cohort comprised 344 women at increased risk for breast cancer due to a strong family history (80%), a genetic mutation (9%) or benign breast disease (16%). Mean IES score was 16.8 (CI 15.5, 18.2), and indicates high distress levels in this cohort. IES scores decreased over time ($p < 0.001$ and $p = 0.003$ after 4 and 8 years, respectively). Scores did not differ between the overall group and either women with a strong family history ($p = 0.06$) or women with BBD ($p = 0.8$). Women with genetic mutations had higher IES scores than those without ($p = 0.023$). IES scores were positively associated with the number of risk factors an individual had (1, 2, or 3 risk factors: $p = 0.003$).

Conclusion: We demonstrated that distress is similar among different risk categories and that distress decreases over time; patterns not previously recognized. Women with benign breast disease have IES scores similar to women with strong family histories of breast cancer. Women with multiple risk factors are also noted to have higher scores. Women with genetic mutations appear to have the highest distress levels. These findings further our understanding of cancer related distress. Targeting women with highest distress levels may improve both quality of life and screening adherence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-11

Title: A phase I safety study of topical calcitriol (BPM31543) for the prevention of chemotherapy-induced alopecia (CIA)

Goldfarb SB B, Konner J, Stevens J, Brouwer S, Narain NR R, Ye R, Ravipaty S, Sarangarajan R, Akmaev VR R, Jimenez JJ Juan, Belum VR Reddy, 'Kitts S, Ciccolini K, Berman B and Lacouture ME E. Memorial Sloan Kettering Cancer Center, New York, NY; Berg, Framingham, MA and University of Miami, Miami, FL.

Body: Background: Chemotherapy induced alopecia (CIA) may lead to significant psychosocial and quality of life issues. Currently there are no FDA approved oral or topical agents available to prevent CIA. In murine studies, topical calcitriol reduced CIA, due to arrest of cell cycle in healthy hair follicles, and reduction in the sensitivity of follicular epithelium to chemotherapy.

Methods: A prospective dose escalation study is being performed in up to 31 women with breast cancer, gynecologic cancer and sarcomas. Each patient is applying 1mL of BPM31543 to her scalp bid, ≥ 5 days prior to initiation of taxane-based chemotherapy for at least 3 months or until the completion of chemotherapy. The study cohorts are: 5/10/20/40/60/80 $\mu\text{g}/\text{mL}$. The first 5 cohorts are completely enrolled and the final cohort is currently being enrolled. Each patient undergoes pk analysis, adverse event (AE) monitoring, patient self-assessment diaries (1-10 scale), and blinded photographic assessments. Efficacy and pK data are still being collected and analyzed for the patients on study, but will be available by December.

Results: Twenty-four subjects have been enrolled so far (evaluatable at this time, $n = 13$). Pk data ($n = 16$; 5-40 $\mu\text{g}/\text{mL}$) showed inter-individual variability, but no significant dose-dependent increase in systemic absorption (range, < 20 -110 pg/mL). Treatment-related AEs (probably/possibly) were mild/moderate in nature and included scalp pain ($n = 1$; 5 $\mu\text{g}/\text{mL}$), elevated vitamin D levels in 1 patient (20 $\mu\text{g}/\text{mL}$) and passage of renal calculus in another ($n = 1$; 40 $\mu\text{g}/\text{mL}$). All subjects reported changes in overall hair fullness, thickness, and volume of hair during chemotherapy. At the 5/10 $\mu\text{g}/\text{mL}$ dose level, $\geq 75\%$ hair loss was reported in 85% of patients. At the ≥ 20 $\mu\text{g}/\text{mL}$ dose level, $\geq 75\%$ hair loss was seen only in 43% of patients. Hair loss/ thinning caused all subjects to change their hair style (onset, week 2; peak, weeks 5-6).

Conclusions: Data have shown that the twice daily application of BPM31543 in patients receiving taxane-based chemotherapy was safe and well-tolerated. Efficacy data from the preliminary analysis was promising and led to the amendment of the study to evaluate two additional higher dose cohorts: 60 and 80 $\mu\text{g}/\text{ml}$.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-12

Title: MOCHA: An institution-based care coordination app for post-hospitalization breast cancer patients

He T, Ogunti R, Yu X, Puppala M, Chen S, Mancuso J and Stephen W. Houston Methodist Hospital, Houston, TX.

Body: Purpose: Hospitals face many challenges in effective care coordination for post-surgery breast cancer patients, especially with scarce resources and limited availability of nurse navigators for care transition and post-hospitalization follow up. Mobile health provides an inexpensive and convenient means of real time care monitoring and communication between patients and care providers. Nevertheless, most current health apps focus on individual consumers and gather information from their daily lives, but do not integrate with clinical workflow or capture physiological and activity data into electronic medical record for real-time monitoring, patient surveillance, and professional care. To fill this gap, we have developed and implemented MOCHA (MethOdist Hospital Cancer Health Application), a coordinated care mobile app for post-hospitalization breast cancer patients from the perspective of a primary care institution. **Methods:** MOCHA supports both iOS and Android platforms and contains two main modules: health care monitoring and data communication, designed together with the physicians and nurses of the Houston Methodist Cancer Center. The Health care monitoring module aims to support real-time monitoring of the post-discharge medical state of breast cancer patients. Physicians can monitor the daily food intake and activities for patients and provide advice to patients in real-time. The data communication module was developed to safely exchange the care coordination data with the hospital electronic medical record or data warehouse. Communication between the patient and the physician can be via an in-house protocol or an open data exchange standard Fast Healthcare Interoperability Resources (FHIR), that describes data format and elements for exchanging electronic health records. Our communication module uses https-based protocol to exchange the structured data with the FHIR resource server. **Implementation:** To validate the MOCHA app, we collaborated with the oncologists and dietitians at the Houston Methodist Cancer Center, who provided breast cancer patients for post-surgery care coordination. Our app exchanges health care data in real time with our hospital's clinical data warehouse. MOCHA searches Nutritionix food database for nutritional information and uses personal trackers such as Fitbit for patients' daily activities with their authorization. The app sends patients' daily burned calories into our clinical data warehouse. During the evaluation period, the physician communicates with cancer patients daily. In addition, every patient has a bi-weekly physical examination, and all examination results are shown in the app. After the experimental evaluation, the physician will access the data warehouse and analyze the test data in order to improve the quality of care coordination. The experimental clinical evaluation is ongoing, and we will report the results once the study is completed. **Conclusion:** MOCHA app provides health care monitoring and secure communication functions with interface with clinical data warehouse. The technical evaluation shows that the proposed methods are robust and efficient in support of care coordination for post-surgery cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-13

Title: Experience in the ABC Medical Center of Mexico City 2010-2015 using scalp-cooling system (DigniCap) for prevention of alopecia induced by chemotherapy

Hernandez RE E, Gerson R, Olivares G, Rivera S, Serrano A, Villalobos A and Lazaro M. Centro Medico ABC, Mexico, Mexico.

Body: Background: Alopecia is one of the secondary side effect whit the most emotionally impact for patients undergoing chemotherapy (CTX) The DigniCap System is the first scalp cooling system use to minimize alopecia. Methods: The objctive of this study was to evaluate in a restrospective trial the efficacy of DigniCap preventing alopecia in consecutive patients treated in ABC Medical Center from December 2010 to January 2015. Patients recieve different chemotherapy regimens, with different modalities, neo , adyuvant and for metastases in first and second line, as in many clinical stages whit breast cancer (BC), were evaluated with the visual scale of Dean (score 0: 0-25%, 1: 25-50%, 2: 50-75%, 3: 75-100%) with photographs of the before and after treatment. Results: 120 pts with BC in stages I-V were treated with a taxane and antraciclins regimen of chemotherapy, 66 pts receive 12 treatment weekly of paclitaxe (T)I and 4 adriamicin/cyclophosphamida (AC) every 21 days, 28 pts 6-8 cycles every 21 days AC-Taxol, 22 pts (18%)suspended the treatment because of the loss of more than 50% of hair at the 2nd an 3rd chemotherapy cycle. 98 pts actually finished the treatment (72%). Of these, 82 pts (84%) no had or had a minimal loss of hair (Dean score 0-1), 16 pts (16%) had a 50% of hair loss (Dean score 3). 8pts recieve more than one regimen of chemotherapy. In the tracing any metastases or side effects were presented with the use of DigniCap. Conclusions: The use of DigniCap minimize alopecia in a 84%, including pts with more than one chemotherapy regimen, in a a safety level.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-14

Title: Physicians perceptions of estrogen agonist/antagonists in menopausal health: An opportunity to address a triad of concerns in menopause and breast cancer survivorship

Krychman M and Portman D. Southern California Center for Sexual Health and Survivorship Medicine Inc, Newport Beach, CA and Sermonix Pharmaceuticals, Columbus, OH.

Body: Introduction: Estrogen agonists/ antagonists (Selective Estrogen Receptor Modulators, SERMS) are a class of medications, which are gaining popularity in menopause health care as they can effectively address a variety of symptoms and offer a non-hormonal alternative. In particular, bone and genitourinary health both suffer from the ramifications of menopause as well as breast cancer treatment. Chemoprevention is also a medical concern in the at risk population. Breast chemoprevention efficacy, and prevention and treatment of osteoporosis as well as improvement of vulvovaginal health are dependent on estrogen receptor activity in the menopause and would be useful targets for treatment of menopausal women, for breast cancer patients and those with genetic predisposition to cancer syndromes who may undergo prophylactic surgeries or face decades of premature menopause and hypoestrogenism. To understand clinician awareness, we conducted an online internet survey to assess the current treatment practices for the use of SERMS for postmenopausal patients, specifically concerning the symptoms of osteoporosis/ osteopenia and vulvovaginal atrophy (VVA). **Methods:** A self- administered Internet survey was conducted for physicians who were potential prescribers of SERMS. One hundred and eight (n=108) physicians were included (OB/GYN n= 53; PCP n= 55). The eligibility criteria included: Primary specialty of OB/GYN or Gynecology for "OB/GYNs," Family Practice, General Practice or Internal Medicine. Physician participants had at least two and not more than 30 years of practice experience. Clinicians were currently in a community-based practice and were known to treat a minimum number of postmenopausal patients per month for both osteopenia/osteoporosis and vulvovaginal atrophy (VVA) **Results:** Physicians in this study saw an average of 100-250 postmenopausal patients per month. Although not statistically significant, OB/GYNs reported a higher monthly average volume of postmenopausal patients (186 patients) versus PCPs (140 patients.) SERMs were prescribed on average for a small percentage of patients though physicians anticipated that this would increase in the future. Nearly half of the physicians (48%) reported that the number of their postmenopausal patients who are receiving prescriptions for medications for prevention of osteoporosis is increasing. A novel SERM under investigation, had features perceived as uniquely favorable by a large majority of clinicians and included: Reducing vertebral fracture by 42% and non-vertebral fracture by 26% (76%), and 80% reduction in incidence of ER+ breast cancers at 5 years (74%), no significant drug-drug interactions (72%), and supported by a 5-year placebo-controlled study (70%). **Conclusions:** Clinicians are aware of the class of SERMS, use is likely to increase and agree that they may be of therapeutic benefit for menopausal women that have multiple post-menopausal health issues. There was recognition of a triad of concerns and the need for concomitant breast cancer chemoprevention, VVA improvement, and maintenance of bone health. Agents that target the estrogen receptor selectively may meet the goals of many clinicians in at-risk patient populations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-15

Title: Rolapitant for the prevention of chemotherapy-induced nausea and vomiting in breast cancer patients receiving multiple cycles of emetogenic chemotherapy

Schwartzberg L, Navari R, Arora S, Powers D, Jordan K and Rapoport B. The West Clinic, Memphis, TN; Indiana University School of Medicine - South Bend, South Bend, IN; TESARO, Inc., Waltham, MA; Martin-Luther-University Halle-Wittenberg, Halle, Germany and The Medical Oncology Centre of Rosebank, Johannesburg, South Africa.

Body: Background: Patients (pts) with breast cancer often receive highly emetogenic chemotherapy, such as anthracycline plus cyclophosphamide (AC). Additionally, young age and female gender are risk factors for chemotherapy-induced nausea and vomiting (CINV) in response to emetogenic chemotherapy. We assessed the ability of the long-acting neurokinin-1 receptor antagonist (RA), rolapitant, in the prevention of CINV over multiple cycles in pts with breast cancer

Methods: This is a post hoc analysis of the prevention of CINV in a subset of pts with breast cancer from 3 similarly-designed, randomized, placebo-controlled phase 3 trials in which pts received a single oral dose of 180 mg rolapitant or placebo before chemotherapy. All pts received an oral 5-HT₃ RA + dexamethasone (active control). The regimens were cisplatin-based (n=36), AC-based (n=681) or other (n=166; carboplatin, cyclophosphamide, etc). Pts who completed cycle 1 could continue the same antiemetic treatment in multiple cycles. Endpoints for cycle 1 of chemotherapy included complete response (CR; no emesis and no use of rescue medication) and no emesis, and no nausea (maximum visual analogue scale [VAS] <5 mm), in the overall (0–120 h), acute (≤24 h), and delayed (>24–120 h) phases. On days 6-8 of each subsequent chemotherapy cycle, pts self-reported the incidence of emesis or nausea interfering with normal daily life.

Results: In cycle 1, CR in both the overall (62.9% rolapitant, 55.1% control; p=0.018) and delayed (66.7% rolapitant, 59.7% control; p=0.032) phases were higher with rolapitant vs control. Rolapitant also improved no emesis rates in the overall (74.4% rolapitant, 62.6% control; p<0.001) and delayed (77.2% rolapitant, 68.5% control; p=0.004) phases. Although less pts were available for follow up over multiple cycles, a numerically greater proportion of rolapitant-treated pts than control pts reported no emesis (cycles 2-6) and no interfering nausea (cycles 2-5) (table). The incidence of treatment-emergent adverse events (TEAEs) was similar for rolapitant (85.2%) and control (83.2%) during cycles 1-6. The most common TEAEs occurred at comparable rates in the rolapitant and control arms: fatigue (28.5% and 29.4%, respectively), alopecia (28.5% and 31.2%, respectively), and constipation (20.0% and 20.9%, respectively).

Conclusions: Rolapitant added to 5-HT₃ RA and dexamethasone therapy improved CINV control and was safe and well-tolerated in pts with breast cancer receiving multiple cycles of emetogenic chemotherapy, mostly AC and carboplatin, historically a high-risk population for CINV.

Cycle	Pt-Reported Response in Multiple Cycles, % (n/N)*					
	No Emesis			No Interfering Nausea		
	Rolapitant 180 mg	Active Control	p-value *	Rolapitant 180 mg	Active Control	p-value *
2	88.3 (323/366)	80.0 (308/385)	0.002	71.6 (262/366)	68.3 (263/385)	0.329
3	90.5 (297/328)	80.5 (293/364)	<0.001	73.8 (242/328)	65.9 (240/364)	0.025
4	87.5 (266/304)	82.4 (277/336)	0.075	79.3 (241/304)	72.0 (242/336)	0.033
5	94.9 (111/117)	88.2 (127/144)	0.059	87.2 (102/117)	80.6 (116/144)	0.152
6	95.3 (102/107)	88.9 (120/135)	0.071	84.1 (90/107)	83.0 (112/135)	0.811

* Unstratified Cochran-Mantel-Haenszel test for between rolapitant and control difference in response rate.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-16

Title: Do patients and nurses outside clinical trial prefer subcutaneous trastuzumab over conventional intravenous infusion?
Instituto Oncologico Nacional experience

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Body: Background In the PrefHer study, subcutaneous administration of trastuzumab was preferred over intravenous administration (iv). In 2014 our hospital approved subcutaneous (sc) trastuzumab for use in patients with early or metastatic Her 2 positive breast cancer. The aim of this study was to evaluate patients (pts) and nurses's preferences in our institution.

Methods Patients with Her2 positive breast cancer and nurses who had received or administered both sc and iv trastuzumab were interviewed to evaluate their preference. Preference for sc was compared with a linear chi squared test.

Results 55 pts were interviewed. Median age was 53 years (30-83). 30 patients in adjuvant/neoad setting and 25 pts in the palliative setting. 41 pts (74.5%) preferred sc 95% CI (63-86) , 3 pts (5.5%) preferred iv 95% CI (-0.5-11.5) and 11 pts (20%) did not have preference 95% CI (9.4-20%) $p < 0.0001$. 72% of pts considered that sc trastuzumab was more convenient ($p < 0.0001$). 11 nurses experienced with both iv and sc trastuzumab administration answered the questionnaire. Median age 41 years (30-55). 10/11 nurses preferred sc administration, 1/10 indicated no preference. All nurses considered that sc was the most convenient administration method for the patients and is highly recommended. The main reason for patients and nurses preference was that sc administration saved time.

Conclusion Patients and nurses prefer s.c over iv trastuzumab. Our results are consistent with PrefHer study. Incorporation of sc trastuzumab could improve quality of care in patients with Breast Cancer Her 2 positive.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-17

Title: Body image in women with breast cancer using a scalp cooling system to reduce chemotherapy induced alopecia

Cigler T, Melin SA A, Klein P, Hurvitz SA A, Melisko M, Moore A, Park GD D, Bageman E, Ver Hoeve ES S and Rugo HS S. Weill Cornell Medical College, New York, NY; Wake Forest School of Medicine, Winston Salem, NC; Icahn School of Medicine at Mount Sinai, New York, NY; University of California Los Angeles, Los Angeles, CA; University of California San Francisco Helen Diller Comprehensive Cancer Center, San Francisco, CA; Target Health Inc., New York, NY and Dignitana AB, Lund, Sweden.

Body: Background: Most women consider hair to be an important part of body image. Alopecia is an emotionally traumatic side effect for breast cancer patients undergoing adjuvant chemotherapy. The DigniCap™ Scalp Cooling System is the first scalp cooling system cleared by the US Food and Drug Administration to reduce the likelihood of chemotherapy induced alopecia.

Methods: Quality of Life (QOL) data were collected as part of a prospective clinical trial evaluating the clinical performance of scalp cooling in women with early stage BC receiving adjuvant chemotherapy.

The study's primary endpoint was hair loss as evaluated by patient self-assessment. Treatment success was defined as $\leq 50\%$ hair loss. QOL was evaluated by the EORTC-QLQ-BR23 (BR23) administered at baseline, last chemotherapy cycle, and one month later. For BR23, 4 response categories were collapsed to 2 categories (Not at all/A little bit and Quite a bit/Very much) for analysis. QOL was compared between those with success vs. failure of scalp cooling.

Results: 101 patients were evaluable for the primary endpoint: Success was seen in 67 (66.3%) pts. QOL at study entry was comparable between pts with scalp cooling success or failure for each item in the BR23 questionnaire. Results reported as percentages of patients in each group who answered either quite a bit or very much to body image-related questions on the BR23 questionnaire are displayed in Table 1.

BR23 results (% quite a bit/very much) one month after chemotherapy

BR23 Items	Treatment Success % (95% CI)	Treatment Failure % (95% CI)
Felt physically less attractive	18.5% (9.0%, 27.9%)	52.2% (31.8%, 72.6%)
Felt less feminine	15.4% (6.6%, 24.2%)	29.1% (19.2%, 59.1%)
Found it difficult to see themselves naked	13.8% (5.5%, 22.2%)	21.7% (4.9%, 38.6%)
Felt dissatisfied with their body	12.3% (4.3%, 20.3%)	26.1% (8.1%, 44.0%)

Conclusions: Women with breast cancer using scalp cooling during chemotherapy who had hair preservation experienced improved quality of life, according to self-assessment of body image, compared to women who had significant hair loss.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-18

Title: Trajectory of patient (Pt) reported physical function (PF) during and after neoadjuvant chemotherapy in the I-SPY 2 trial

Shah M, Jensen R, Yau C, Straehley I, Berry DA A, DeMichele A, Buxton MB B, Hylton NM M, Perlmutter J, Symmans WF Fraser, Tripathy D, Yee D, Wallace A, Kaplan HG G, Clark A, Chien AJ Jo, I-SPY 2 Investigators, Esserman LJ J and Melisko ME E. University of California, San Francisco, San Francisco, CA; Georgetown Lombardi Comprehensive Cancer Center, Washington, DC; Berry Consultants, Austin, TX; University of Pennsylvania, Philadelphia, PA; Gemini Group, Ann Arbor, MI; MD Anderson Cancer Center, Houston, TX; University of Minnesota, Minneapolis, MN; University of California, San Diego, San Diego, CA; Swedish Meidcal Center, Seattle, WA and QuantumLeap Healthcare Collaborative, San Francisco, CA.

Body: Background

Patients (pts) receiving chemotherapy for breast cancer experience toxicities impacting short and long-term quality of life (QOL). Within I-SPY 2, a trial adaptively randomizing stage II/III breast cancer pts to neoadjuvant chemotherapy +/- an investigational agent, we are collecting pt reported outcome (PRO) data to understand the impact of investigational agents on QOL. This PRO sub-study provides a unique opportunity to study QOL longitudinally and explore how pt and tumor characteristics, exposure to investigational therapies, and surgical outcome impact QOL.

Methods

Pts enrolled in this trial receive paclitaxel (T) +/- an investigational agent for 12 weeks followed by 4 cycles of doxorubicin and cyclophosphamide (AC). Surveys include the EORTC QLQ-C30 and BR-23, and PROMIS measures for QOL metrics including but not limited to physical function (PF), anxiety, and depression. Surveys are administered pre-chemotherapy to 2 years post-surgery. PF data from the EORTC and PROMIS instruments was analyzed for 238 pts at 5 sites (UCSF, UCSD, U of Pennsylvania, U of Minnesota, and Swedish Cancer Center). 48 pts completed baseline, inter-regimen (between T and AC), pre-operative and post-surgery surveys. Of the 48 pts 32 completed a 6-month follow up (FUP) and 31 completed a 1-year FUP survey. A linear mixed effect model, adjusting for HER2 status and treatment type was used to evaluate changes in PF over time. Sample size is small and statistics are descriptive rather than inferential.

Results

Median age of pts in this analysis was 50 (range 27-72).

Table 1 shows PROMIS & EORTC PF scores in this cohort.

Time Point		n	PROMIS		EORTC	
			Mean	SE	Mean	SE
Pre-Treatment	All	48	52.5	1.0	92.0	2.0
	HER2+	15	53.5	1.5	94.1	2.2
	HER2-	33	52.1	1.3	91.1	2.8
Inter-Regimen	All	48	45.5	1.2	82.2	2.7
	HER2+	15	48.6	2.3	84.4	4.2
	HER2-	33	44.1	1.3	81.2	3.4
Pre-Surgery	All	48	43.9	1.1	79.4	2.3
	HER2+	15	45.1	2.2	75.3	4.1
	HER2-	33	43.4	1.3	81.3	2.8
6-Month FUP	All	32	48.1	1.4	87.4	1.9
	HER2+	12	47.5	2.2	85.0	3.3

	HER2-	20	48.4	1.8	88.9	2.4
1 Year FUP	All	31	48.9	1.4	88.4	3.1
	HER2+	9	49.1	2.9	88.9	5.4
	HER2-	22	48.8	1.7	88.3	3.8

At baseline, mean PROMIS PF scores were higher than the US average (mean = 50) but declined as expected throughout treatment. HER2+ patients experienced a similar degree of recovery as HER2- pts post-surgery despite adjuvant treatment with Herceptin. Analysis of post-operative PROMIS PF indicated an average score within the U.S. general population (mean =50) but did not return to higher functioning seen at baseline levels (mean 52.5, p-value < 0.05). Analysis of the EORTC PF sub-scale demonstrated a similar trend; however, the baseline and post-operative difference was not significant (p-value=0.15 for both FUP). Finding supports PROMIS PF ability to measure high functioning cancer patients.

Conclusions: Among a subset of pts who completed all surveys in the I-SPY 2 QOL substudy, PF did not return to baseline at 6-12 months post-operatively. Through transition to an electronic platform of data collection we hope to improve compliance with survey completion. We continue to analyze other QOL measures and plan to correlate QOL data with treatment arm, adverse events, comorbidities, and response to neoadjuvant treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-11-19

Title: Sexual functioning among breast cancer survivors and their partners: A pilot study

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Body: Introduction: Although a number of studies have examined sexual difficulties among breast cancer survivors, few have focused on multiple dimensions of sexual functioning or partners' attitudes and difficulties. This pilot study sought a more comprehensive examination of sexual functioning among couples after breast cancer.

Methods: Patients who were at least 6 months post-treatment and were involved in committed relationships were recruited during routine clinic visits. Those who accepted to participate in the study were handed by the investigator a stamped self-addressed envelope (to the research office of the Cancer Institute) containing two questionnaires, for the patient and for her partner, and a cancer information sheet. No identifiable information was collected. Thus, the IRB did not require a written consent form.

Results: 200 patients were invited to participate in the study. 120 were in a stable relationship but only 100 accepted to take the envelope. The rest of the patients were single (76) or separated after their diagnosis (4; 2%). 38 couples participated by returning their completed questionnaires; they had been partnered for an average of 34.9 (14.3) years. Mean time since diagnosis was 40.4 (22.4) months, and mean age was 58.1 (11.5). 71.05% were currently receiving hormonal therapy.

Among the patients, 63.2% reported that they were sexually active and, retrospectively, reported that their sexual relationship had been satisfying prior to diagnosis. Currently, however, a large subgroup of patients (30.4%) reported markedly limited sexual satisfaction ("a little bit" or "not at all"). Additionally, 30.4%-to-43.5% reported low sexual interest; 69.6% reported significant problems with lubrication; 52.2% reported limited orgasm; and 30.4%-to- 60.9% had difficulties with vaginal discomfort.

Among partners, 67.6% reported that they were sexually active. Their sexual relationships prior to diagnosis were recalled as having been highly satisfying. Currently, none of the sexually active partners reported low satisfaction, though a few (8.3%) had not been active in the past month. Few partners reported low sexual interest (0% - 4.2%). Marked erectile difficulties were reported by 16.7% to 20.8%, and 25.0% reported difficulties with orgasm.

The top 3 factors that patients endorsed as interfering with their current sexual functioning ("quite a bit" or "very much") were (1) pain (41.7%), (2) fatigue (37.5%), and (3) feeling unattractive (20.8%). Fewer participants endorsed concerns about breast tenderness (16.7%), hot flashes (12.5%), surgical scars (12.5%), their partners' reactions (16.7%), and their partners' own sexual difficulties (12.5%). For partners, the factors most commonly viewed as disrupting the sexual relationship were the patient's vaginal dryness (32.0%), the patient's fatigue (28.0%), and the partner's own sexual problems (20.0%).

Conclusion: Our results suggest that a large proportion of breast cancer survivors experience difficulties in multiple dimensions of sexual functioning. Contrary to a popular belief, separation rate after the diagnosis of breast cancer was low (2%) and most patients and their partners identified vaginal dryness and fatigue (not the breast surgery) as the major factors interfering with their sexual functioning.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-12-01

Title: Randomized trial of exercise intervention vs. usual care for breast cancer patients with aromatase inhibitor to prevent and improve the aromatase inhibitor induced arthralgia

Tajaesu M, Tamaki K, Nagamine S, Kamada Y, Uehara K, Arakaki M, Tamatsu Y, Yamashiro K, Miyashita M, Ishida T, Ohuchi N, McNamara K, Terukina S, Sasano H and Tamaki N. Nahanishi Clinic Okinawa, Naha, Okinawa, Japan and Tohoku University, Sendai, Miyagi, Japan.

Body: Purpose: Arthralgia sometimes occurs in the breast cancer patients treated with aromatase inhibitors (AIs). It is one of the most important reasons for poor AIs adherence.

Background; The HOPE study previously demonstrated that exercise was effective in improving AI-induced arthralgia in breast cancer patients. However, recruitment to this study was limited to severe cases (Criteria; physically inactive, >6 months AI treatment, >2 months arthralgia). To assess if these findings were more generalizable to all breast cancer contexts we conducted a randomized trial of exercise intervention using wider eligibility criteria than the HOPE study to assess the impact on AI induced arthralgia in breast cancer patients.

Methods: We examined Japanese breast cancer patients operated at Nahanishi Clinic, Okinawa, Japan. Following the informed consent the patients were randomly assigned to a 3:1 ratio to exercise intervention or usual care. Eligibility criteria included receiving an AI for 0-4 years, no metastases, any arthralgia level and any exercise habits. Following randomization participants could choose from 3 types of exercise including strong (120-150 minutes per week of walking or running), intermediate (gentle calisthenics (daily NIPPON HOSO KYOKAI: NHK broadcast exercise)) and weak (going up the stairs- frequency). Arthralgia was assessed using the Brief Pain Inventory (BPI), in which the patients completed a baseline, 6 month and 12 month BPI assessment. Primary endpoint was BPI change at 12 months.

Results: Among 227 women screened, we randomized 108 women, with 80 to exercise intervention (46 of strong, 19 of intermediate and 15 of weak) and 28 to usual care. Base line BPI were well balanced between exercise intervention and usual care. Overall exercise intervention reduced BPI scores relative to control. The BPI changes of worst pain, least pain, average pain and pain right now were 0.09, -0.25, -0.14 and 0 for exercise intervention group and 0.21, 0.46, 0.07 and 0.61 for usual care group, respectively. There was a statistically significant difference of AIs adherence between exercise intervention group (99%) and usual care group (92%) (P=0.03).

Conclusion: Exercise intervention tends to improve the AI-induced arthralgia and has a positive effect on AIs adherence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-12-02

Title: Can a supervised exercise program compared to usual care prevent aromatase inhibitor-induced musculoskeletal pain in women with breast cancer?

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Body: Background: Aromatase Inhibitors (AIs) are commonly prescribed as hormone therapy for post-menopausal women with estrogen receptor-positive breast cancer. A number of authors have reported AI side effects that include increased risk for developing osteoporosis, joint pain, weight gain and depression. For example, 30-40% of post-menopausal women treated with AIs experience mild to severe joint pain. Although the exact causes of AI-induced joint pain are unknown, the side-effects are most likely due to low estrogen levels in the body. . Denysschen et al. (2014) reported a significantly lower number of painful joints, reduced depressive symptoms, increased muscle strength, and improvement in quality of life in patients with AI-induced joint pain after an 8 week upper and lower body resistance exercise program. **Objective:** To investigate whether a supervised exercise program could reduce or prevent musculoskeletal pain in patients with breast cancer undergoing AI therapy. **Methods:** Twenty participants with breast cancer have thus far been randomised to either: (a) usual care and advice regarding benefits of regular exercise; or (b) usual care + 12 week supervised and home-based exercise program consisting of upper and lower body resistance exercises with self-selected aerobic exercises. Participants accrued 150 mins / week of moderate intensity aerobic exercise at 60-70% HR_{max} on 5 or more days of each week including 2 supervised sessions / week. Initial exercise intensity is individualized and generally begins at 55% to 60% of HR_{max} (15 to 30 minutes per session) and progresses to 60% - 70% of HR_{max} by week 6. Strength training consisted of 2 supervised sessions per week, of two sets of 10 to 12 repetitions of eight different strength exercises at 60% to 75% of estimated one repetition maximum. On at least 3 other days, using the same dosing principles participants executed 4 lower and 4 upper body resistance exercises at home using resistance bands. The primary outcome measures were pain (brief pain inventory; BPI) scores and grip strength (JAMAR dynamometer (kg)) measured at baseline, 3, 6 and 12 months. Two-way repeated ANOVAs were undertaken to evaluate differences between groups and factors. **Results:** Mean pain scores across 12 months were largely maintained ($p>0.05$) for participants undertaking the exercise program compared to control participants whose mean BPI scores progressively increased from baseline through to 12 month follow-up. In the exercise group, mean pain scores increased by one BPI unit between baseline and 12 month follow-up. In the control group, mean pain scores increased by five BPI units between baseline and 12 month follow-up. A clinically significant difference in BPI scores is a change of two or more BPI units (Mease et al, 2011). Grip strength measures were significantly different ($P<0.001$) between groups at each time point with a trend towards improved grip strength between baseline and 6 months in the exercise group. Grip strength decreased in both groups between 6 and 12 months. **Conclusion:** By managing physical performance and pain as side effects of AIs, a 12 week supervised exercise program may contribute to preventing non-adherence with or withdrawal from AI therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-01

Title: Sociodemographic and economic factors are essential determinants of weight gain between before and after cancer diagnosis: Results from the prospective population-based NutriNet-Santé cohort

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Body: Background: While many cancer patients are affected by weight loss, others tend to gain weight, which may impact prognosis and risk of recurrence and of second cancer. The aim of this prospective study was to investigate weight variation between before and after cancer diagnosis and socio-demographic, economic, lifestyle and clinical factors associated with moderate-to-severe weight gain.

Methods: 1051 incident cases of first primary cancer were diagnosed in the NutriNet-Santé cohort between 2009 and 2015. Weight was prospectively collected every 6 months since subjects' inclusion (i.e. an average of 2y before diagnosis). Mean weights before and after cancer diagnosis were compared with paired Student's t-test. Factors associated with moderate-to-severe weight gain ($\geq 5\%$ of initial weight) were investigated by multivariable logistic regression.

Results: Weight loss was observed in men (-3.54kg in those who lost weight, $p=0.0002$) and in colorectal cancer patients (-3.94kg, $p=0.0012$). Weight gain was observed in breast and skin cancers (2.83kg, $p=0.047$, and 2.96kg, $p=0.03$ respectively). Women (OR=1.99[1.18-3.35]), younger patients (OR=1.78[1.05-3.03]), those with lower education (OR=2.17[1.07-4.37]), those with excess weight before diagnosis (OR=1.53[1.02-2.30]) and those who stopped smoking after diagnosis (OR=4.60[2.06-10.25]) were more likely to experience moderate-to-severe weight gain. In breast cancer patients, induced menopause was associated with weight gain (OR=4.12[1.76-9.67]), but no association was detected for tumor characteristics or treatments.

Conclusion: This large prospective cohort provided original results on weight variation between before and after cancer diagnosis, highlighting different weight trajectories. Socio-demographic and economic factors appeared to strongly influence the risk of weight gain, illustrating social inequalities in health.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-02

Title: Weight gain after breast cancer diagnosis and patients' opinion on weight loss strategies

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Body: Background: The rate of obesity is increasing in many countries worldwide. Most populations are not aware of steady weight gain with age. In addition, obesity is increasingly being recognized as a risk factor for breast cancer. Some studies have also demonstrated that weight gain after diagnosis is associated with increased risk for recurrence. The current study was performed to evaluate weight gain in a population of patients seen at a safety net institution. Additionally, patients also gave their opinion on weight loss strategies.

Methods: A retrospective review of all breast cancer patients seen at the county, safety net institution from July 2001 to June 2014 who had at least 2 years of follow up were evaluated for change in weight. For the question on weight loss strategies, all patients from May 2014 to May 2015 were included. Sociodemographic, clinical, and treatment variables were evaluated.

Results: From July 2001 to June 2014, 225 breast cancer patients had follow up prior to January 2013. Of these patients 59% gained weight after their diagnosis of breast cancer. Overall these patients gained an average of 2 kg after their diagnosis.

Starting in January 2013, patients were given a simple message, "avoiding gaining weight" after their diagnosis. For 115 patients with follow up after January 2013, only 35% gained weight and on average, this group of patient lost 4 kg from diagnosis. Patients who initially gained weight were provided with basic weight loss strategies.

From May 2014 to May 2015, 1198 consecutive patients were seen. The average age was 45 years. 12% were non-Hispanic White. Only 30% of patients stated that their primary care provider discussed weight maintenance or weight loss as part of their routine health care. 40% of patients did feel that a permanent change was necessary to facilitate weight loss. However, fewer than half (44%) the patients felt that exercise was necessary. Dietary beliefs were varied, with the most commonly held belief was that vegetarian/vegan diet was necessary (28%). Patients rarely cited commonly recommended weight loss strategies: lower caloric intake (9%), eat frequently (0.3%), adequate fiber (0.1%), adequate protein (0.6%), enough sleep (0.1%), don't eat late (0.1%), no fast food (2%), avoid soda (1%), drink more water (1%). 6% of patients felt a low/no carbohydrate diet was important. By contrast, older strategies or popular ideas: low fat (8%), no flour/wheat/gluten (4%) were also felt to be important. 7.3% stated they did not know any strategy. 5% patients recommended stopping eating altogether to lose weight.

Conclusions: Similar to population wide data, breast cancer patients treated at a safety net institution tend to gain weight with follow up. Also similar to most populations, the population was not aware of recommended weight loss strategies and most do not feel that exercise is an important component of weight maintenance. As with the rest of the US population significant effort will be necessary to help patients avoid weight gain after diagnosis. Making patients aware of their weight may help patients avoid gaining weight.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-03

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-04

Title: Voice of cancer patients: Analysis of patient concerns regarding pregnancy and fertility preservation in patients with breast cancer

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Body: Breast cancer accounts for one third of all cancers seen in reproductive-age women. Adjuvant chemotherapy regimens used for treatment commonly affect fertility and cause premature ovarian failure. Fertility preservation in young cancer survivors is recognized as a key survivorship issue by the American Society of Clinical Oncology and the American Society of Reproductive Medicine. Many patients share their experiences in online forums, which contain millions of freely shared messages that can be used to analyze concerns regarding pregnancy and fertility preservation. However, this data is unstructured and difficult to analyze. We organize it using methods from Big Data Science (BDS) and analyze it by creating a Decision Support System (DSS) and an interface that can be used by patients and by providers to understand concerns of these patients.

Method:
We collected 5.8 million unique messages (by 170,000 users) from 20 unrestricted breast cancer forums that provide clinically relevant information. We next built custom ontologies for breast cancer, treatment, pregnancy and fertility and supportive therapies. We then created a DSS using methods from BDS, including topic modeling, information retrieval, and natural language processing to extract relevant information from these messages.

To use this system, a user provides disease-related parameters, key questions and the treatment. The DSS then gives messages discussing a similar cohort of patients that have similar parameters and are going through similar treatments.

Results:
Of 170,000 patients, only 5,109 patients reported their age and 1,604 patients reported their age below 45 years. 2,378 patients posted 6,377 messages discussing pregnancy and fertility related issues during Breast-Cancer treatment, and of these, 907 reported their age below 45.

Their specific concerns included:

- Does pregnancy increase the risk of recurrence (31 messages from 24 patients)
- Chances of successful pregnancy in patients who are 30 to 39 years old
- Risk of birth and genetic defects (23 messages from 21 patients)

Fertility options being discussed:

- Freezing embryos, eggs, or ovarian tissues. (169 messages from 138 patients)
- Artificial Insemination, hormone assisted pregnancy, or ovarian stimulation- in vitro fertilization (55 messages from 43 patients)
- Use of GNRH to preserve fertility during chemo with Lupron (291 messages) and Zolodex (391 messages)

Other findings include:

- 893 messages talked about stopping tamoxifen early, mostly within first 1.5 to 3 years so as to try the pregnancy option and 116 patients said that they stopped tamoxifen early.
- 83 patients talked about a successful pregnancy. Only 40 patients discussed getting fertility experts involved early.

Conclusion:
· This interactive system reliably provides meaningful insights from patient's point of view to their treatment, their concerns, and suggestions for supportive therapy. It also gives insight into unmet needs where more resources and research should be focused.
· Fertility preservation is a major concern in pre-menopausal patients. It should be addressed early on to prevent patients taking harmful decisions such as cutting short their hormonal therapy.
· Fertility counseling seems to be underutilized.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-05

Title: Effect of aromatase inhibitor (AI) therapy on sleep and activity patterns in early stage breast cancer

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Body: Background: AIs are commonly used for treatment of postmenopausal women with hormone receptor-positive breast cancer for 5-10 years. However, women often experience side effects including musculoskeletal pain and sleep disturbances that lead to treatment discontinuation and worse healthcare-related outcomes. There is a need to understand the impact of adjuvant AI therapy on sleep and activity patterns in treated patients and associations with treatment adherence. This study examines objective and subjective changes in sleep patterns and daytime function in patients starting AI therapy using actigraphy and questionnaires.

Methods: 49 postmenopausal women with hormone receptor-positive breast cancer who completed local surgery and chemotherapy and were planning to start AI therapy were enrolled. Patients completed 10 consecutive days of actigraphy and validated questionnaires to assess pain, sleep quality, fatigue and physical function at baseline and after three months of AI therapy. Changes in actigraphy parameters after 3 months of AI therapy were examined using paired T-tests and associations between actigraphy measures and patient-reported outcomes were examined using Pearson's correlations.

Results: 42 patients completed the baseline assessment and 22 patients completed the 3 month assessment. 20 patients were excluded due to incomplete actigraphy data (n=3), discontinuation of AI therapy (n=5) or withdrawal from the study per patient preference (n=12) prior to the 3 month assessment. Total 24 hour activity, average activity per minute and maximum activity over a 24 hour period worsened after 3 months of AI therapy (all p-values <0.05).

Changes in actigraphy variables and questionnaires after 3 months of AI therapy

Mean actigraphy sleep and activity variables	Baseline assessment	3 month assessment	P-value*
24 hour total activity	212590	188104	0.019
24 hour average activity per minute (AC)	157.6	141.0	0.037
24 hour maximum activity (AC)	1035	903	0.012
Total sleep time (min)	456.1	469.1	0.17
Sleep efficiency (%)	77.2	74.6	0.068
Wake after sleep onset (min)	72.9	82.0	0.52
Total activity at night (AC)	5953	6705	0.13
PROMIS Physical Function	52.1	48.3	0.001
Brief Pain Inventory Severity	0.60	1.1	0.10

*p-value from t-test

There was a trend towards worse sleep efficiency after 3 months. Patient-reported physical function also decreased after 3 months of AI therapy (p-value <0.05), but pain severity remained unchanged. Worsening patient-reported fatigue after 3 months of AI treatment correlated to a decrease in average activity per minute ($r=-0.55$, p-value <0.05) and maximum activity over a 24 hour period ($r=-0.61$, p-value <0.05).

Conclusions: Daytime activity and sleep patterns are negatively affected by AI therapy and are associated with worsening fatigue. Future research is needed to better understand the relationship between fatigue and daytime activity, which may lead to interventions to improve tolerance and adherence to AI therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-06

Title: Factors associated with follow up medical care among women with early stage breast cancer

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Body: Introduction: In patients with early stage breast cancer treated with curative intent, optimal follow-up guidelines vary widely among national organizations. NCCN guidelines suggest patients should be followed by a medical oncologist (MO) every 3-6 months and by a radiation oncologist (RO) every 6-12 months for the first 5 years. These recommendations are not evidence based and have an unknown effect on cancer outcomes. We sought to evaluate the patterns of follow-up care and predictors of discontinuation of follow-up care.

Methods: Using the Surveillance, Epidemiology, and End Results (SEER)-Medicare linked dataset, we evaluated patients diagnosed with stage I and II breast cancer who underwent surgery from 2002-2007 with follow-up to 2012. Patients who died in the 5-year period following diagnosis were excluded. We evaluated patterns of follow-up visits for the 5 years after diagnosis among surgeon, MO and RO. We defined discontinuation of follow-up care as >12 months without a visit claim from any of the three providers. We performed a Cox-proportional hazards multivariate analysis to determine factors associated with discontinuation of follow-up care. Patients were censored if a new cancer was diagnosed.

Results: A total of 35,484 stage I and II breast cancer patients were included in the analysis. In addition to the surgeon, 77.5% saw a MO, and 57.8% saw RO in the first year. The mean number of total physician appointments for years 1-5 were 9.4, 3.3, 2.4, 2.0 and 1.7, respectively. During the 5 years, 13,908 (39.6%) patients discontinued follow-up visits. The discontinuation rate averaged about 12% per year for years 2-5. Discontinuing physician visits increased with increasing age. Patients who saw all 3 physicians in year 1 were less likely to discontinue follow-up visits (OR = 0.54, 0.51-0.57). Patients were more likely to discontinue physician visits if they were hormone receptor negative (HR = 1.41, 1.33-1.49), were black (HR = 1.14, 1.06-1.22) or Hispanic (HR = 1.36, 1.17-1.58) compared to white, lived in a rural as opposed to urban setting (HR 1.12, 1.05-1.18), were unmarried (HR = 1.16, 1.12-1.20), had a higher comorbidity score (HR = 1.15, 1.10-1.21), or were in a lower SES quintile (HR = 1.08, 1.02-1.15). Women who had a mastectomy (vs lumpectomy) (HR = 0.83, 0.80-0.87) and those who were receiving chemotherapy (HR = 0.55, 0.52-0.59) or radiation therapy (OR = 0.60, 0.57-0.62) were less likely to discontinue physician visits.

Conclusions: Clinical practice guidelines for surveillance of breast cancer after primary treatment are based on expert opinion and have an unclear effect on long-term outcomes. Coordination of follow-up care may reduce discontinuation. More research is needed to determine the optimal follow-up for maintaining adherence to therapy, reducing over-testing and encouraging secondary cancer screening guidelines.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-07

Title: Conditional disease-free survival among patients with breast cancer

Paik H-J, Lee SK, Park S, Ryu JM, Kim I, Bae SY, Yu J, Lee JE, Kim SW and Nam SJ. Samsung Medical Center, Seoul, Korea.

Body: Background: Conditional disease-free survival (CDFS) reflects changes over time. Because traditional disease-free survival (DFS) is estimated from the date of diagnosis, it is limited in the ability to predict risk of recurrence in patients who have been disease free. In this study, we determined CDFS of breast cancer patients and estimated the prognostic factors for DFS.

Method: We retrospectively reviewed clinical data of 7587 consecutive patients who underwent curative surgery for breast cancer between January 2004 and December 2013 at Samsung Medical Center. Univariate and multivariate analyses were performed to identify risk factors for DFS, which was computed using the Kaplan–Meier method. CDFS rates were based on cumulative DFS estimates.

Results: Median follow-up duration was 20.44 months. Three-year DFS was 93.46 percent at baseline. Three-year CDFS survival estimates for patients who had been disease free for 1, 2, 3, 4 and 5 years after treatment were calculated as 92.84, 92.37, 93.03, 89.41, and 79.64 percent, respectively. Three-year CDFS increased continuously each year after 1 year of DFS in hormone receptor (HR)-negative patients but decreased each year in HR-positive patients.

Conclusion: In HR-positive patients who are disease free after 3 years, continuous care including surveillance and metastases workup should be considered, although this is not recommended in the current guidelines. On the other hand, the social costs may be reduced in HR-negative patients by extending the surveillance interval. Further studies are needed to identify indicators of DFS prognosis in breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-08

Title: The perception of chronic pain among breast cancer survivors

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Body: Introduction: Breast cancer treatments may result in acute pain that, if untreated or undertreated, may lead to chronic pain. Twenty to thirty percent of patients who undergo mastectomy experience post-mastectomy pain syndrome, and up to 50% of breast cancer survivors taking aromatase inhibitors (AI) report aromatase inhibitor associated arthralgia (AIAA). The rates, experiences, and risk factors of the effects of breast cancer treatment on breast cancer survivors' perceptions of chronic pain are not well defined.

Methods: We conducted a cross-sectional survey among postmenopausal, hormone receptor-positive, breast cancer survivors who were currently taking or had previously taken an AI. The main outcome variable was the patient's perception of living with chronic pain. Variables including breast cancer treatment, socioeconomic and clinical factors, symptom burden, pain (measured by the Brief Pain Inventory [BPI]), and pain-related coping (measured by the Pain Catastrophizing Scale [PCS]) were measured. Univariate and multivariate logistic analyses were performed to identify the risk factors associated with patients' perceptions of living with chronic pain.

Results: Among 561 participants, 62 (11.1%) reported that they perceived themselves as living with chronic pain prior to their breast cancer diagnosis and 172 (30.8%) reported living with chronic pain during the past 6 months at the time of the survey. Compared with patients who did not perceive chronic pain, patients with the perception of chronic pain had significantly higher pain severity (4.15 ± 0.17 vs. 1.54 ± 0.09) and interference (3.47 ± 0.2 vs. 0.97 ± 0.08); higher pain medication usage (percentage of patients taking at least one pain medication over the past 7 days: 85.47% vs. 57.36%); and higher levels of negative coping with pain, including rumination (4.08 ± 0.31 vs. 2.28 ± 0.16), magnification (2.27 ± 0.2 vs. 1.15 ± 0.08), and helplessness (4.63 ± 0.37 vs. 1.64 ± 0.13). Among participants, 369 (65.78%) reported taking at least one pain medication over the past 7 days. In a multivariate analysis, age (<56 or >70), higher BMI (>30), prior chemotherapy, currently experiencing AIAA, and the presence of chronic pain before cancer diagnosis were identified as risk factors associated with the perception of living with chronic pain.

Conclusion: One in three breast cancer survivors considered themselves to be living with chronic pain, and two-thirds developed such perceptions following their cancer diagnosis. Both chemotherapy and AI use were risk factors for perceptions of chronic pain. Those women who had chronic pain experienced greater pain severity, daily interference, and more negative coping. Better understanding of the risk factors and symptom burden associated with chronic pain perceptions among this population may allow for more targeted interventions to reduce chronic pain and its negative sequelae, including premature termination of AI treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-09

Title: Development and implementation of a patient-centered, nurse practitioner-led survivorship intervention for breast cancer

Post KE E, Moy B, Furlani CM M, Smith BL L, Strand EA A, Taghian AG G and Jeffrey P. Massachusetts General Hospital, Boston, MA.

Body: Background: Despite a call for action to improve survivorship care, there is no consensus about the optimal model of care delivery. Challenges include time and resources required for an independent survivorship clinic and conflicting evidence regarding the value of survivorship care plans (SCP). In this context, we sought to develop and evaluate the feasibility of a model of nurse practitioner (NP) led survivorship visits (“bridge visits”) within the breast cancer clinic following completion of initial therapy.

Methods: Patients received a NP-led Survivorship Bridge visit (NPSBV) following treatment for early stage breast cancer. Using an embedded care design, oncology NPs met with patients within six months after treatment and collaborated to create a SCP. We conducted a cross-sectional self-administered paper survey evaluating patient satisfaction with the NPSBV and overall care and a parallel survey of patients presenting for routine follow-up as a control population.

Feasibility was defined as greater than 75% NPSBV attendance rate and no difference in satisfaction with care compared to control patients. Differences between groups were assessed with chi-square test and two-tailed p value at 0.05 percent.

Results/Conclusion: Overall, 117 of 166 surveys were returned for a 71% response rate (RR). Among the NPSBV patients, 72 of 109 surveys were returned (66% RR). Of the control patients, 45 of 57 surveys were returned (79% RR). 64% of NPSBV patients were aged 55 or older, 96% were White, 4% were Asian and 1% identified as Hispanic or Latino. In the control group, 50% were aged 55 or older, 83% were White, 9% Black, 6% Asian, 1% other and 2% identified as Hispanic or Latino. Satisfaction with care was high for both groups. 95% of participants reported that their providers always had respect for what they had to say, 94% reported that providers always spent enough time with them and 94% reported that they always trusted their providers with their medical care. NPSBV patients were more likely to report that their team “always” cared as much as they do about their health compared to control patients (91% vs. 80%, $p = .0060$). There were no other significant differences between groups.

The NPSBV met feasibility with a 99% attendance rate and had high acceptability and satisfaction rates. 94% reported that the NPSBV provided easy to understand information. 94% reported that the NPSBV helped to explain the follow up plan, 97% reported it provided helpful information on lifestyle/wellness and 99% reported that they would recommend the NPSBV to other patients. However, 4% felt that their emotional/spiritual needs were not met by the NPSBV and 14% felt that their emotional/spiritual needs were only somewhat met. Further, 18% felt that the NPSBV only somewhat resolved baseline concerns regarding breast cancer survivorship care (BCSC).

Implications: A patient-centered NPSBV for breast cancer survivors is feasible and associated with high patient satisfaction. Findings will guide the refinement and implementation of this embedded care design for BCSC delivery. Future studies should evaluate how survivorship interventions can impact baseline concerns and how best to tailor survivorship interventions to each individual.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-10

Title: A patient centered intervention for adaptation of cancer risk reduction guidelines in breast cancer survivors

Thomas PS S, Klimitchek A, Nelson B, Damani S and Bevers TB B. University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Cancer survivors are susceptible to cancer recurrence and other cancers and this is an opportune time to focus on cancer prevention. The American Institute of Cancer Research (AICR) and the American Cancer Society estimate that modifications to the behaviors of diet and physical activity in addition to maintaining a normal body mass index (BMI) can help reduce cancer incidence by one third. The AICR and the American College of Sports Medicine (ACSM) have established guidelines on nutrition and physical activity respectively for cancer risk reduction. Cancer patients' transition to survivorship is an ideal time for them to make lifestyle modifications and adopt these guidelines. Although studies have evaluated structured exercise regimens and nutritional interventions, data is lacking on how to best achieve long term lifestyle changes in this population. By introducing a patient-centered intervention that utilizes motivational interviewing to our breast cancer survivors, we plan to increase the rate of adherence to the AICR and ACSM guidelines through tailored counseling focused on patient-driven lifestyle modifications.

Methods: Breast cancer survivors 5 years out from their diagnosis seen in the survivorship clinic were also scheduled to be seen by a health education specialist (HES) for a baseline visit. One year follow up visits were scheduled with the HES for reassessment of their nutrition and physical activity goals. All patients completed a survey of questions assessing their adherence to the nutritional and physical activity guidelines on cancer risk reduction at baseline and at 1 year follow up visit. Anthropometric measures including height, weight, BMI, pulse, respirations, and blood pressure were collected at each visit with their provider. Baseline demographic data was collected on all participants including age, race/ethnicity, cancer stage, previous cancer treatment(s), and comorbid conditions. Frequency of interactions (i.e clinic visit, telephone call, or secure message) with the HES were extracted from the medical record.

Results: From October 6, 2014 to May 31, 2016, 200 breast cancer survivors were seen in the survivorship clinic and by a HES. Fifty women who completed a baseline and 1 year follow up survey were selected to assess their adherence to the AICR and ACSM guidelines on cancer risk reduction. Sixty percent of patients decreased their BMI, 5% maintained their BMI, and 35% had an increase in BMI after one year follow up. There was an increase in the percentage of patients adhering to all of the guidelines after one year of follow up in comparison to baseline. There were notable increases in adherence to physical activity guidelines with 24% of survivors achieving at least 150 minutes of moderate exercise each week at baseline assessment and 34% of survivors meeting this guideline at the 1 year follow up visit.

Discussion: Through our intervention with a HES in our survivorship clinic, we were able to see trends toward increased adherence to the AICR and ACSM guidelines. Further analysis is ongoing to assess the frequency of interactions with the HES and their adherence to the guidelines. This analysis will give us a better understanding of the ideal target population for this intervention.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-12

Title: Symptom burden, unmet need for assistance, and psychosocial adaptation among longer term breast cancer survivors

Palmer SC C, Blauch AN N, Pucci DA A and Jacobs LA A. University of Pennsylvania, Philadelphia, PA.

Body: Introduction:

Advances have improved survival among breast cancer (BC) patients and 89% can expect >5 year survival. The price for this survival, however, may be physical and psychosocial symptoms that persist many years into the survivorship trajectory. Unmet needs for symptom management and the relationships between unmet needs, symptom burden, and psychosocial adaptation remain unclear. We examined these relationships among longer term BC survivors.

Method:

Eligibility included non-metastatic BC survivors diagnosed > 3 years prior and attendance at a survivorship-focused appointment. Nineteen common symptoms of disease and treatment were evaluated and participants reported unmet need for assistance for each symptom they experienced. Psychosocial adaptation was assessed through the Hospital Anxiety and Depression Scale (HADS).

Results:

103 primarily white (72%), middle aged (M=62.7 yrs) BC survivors were recruited. Participants were, on average, 11.4 yrs from diagnosis and most (78.2%) reported Stage I or II BC. Participants reported an average of 9.2 symptoms, most commonly fatigue (67%), joint pain (66%), weight gain (60%), decreased sexual drive (55%), and insomnia (52%). Participants reported an average of 2.8 unmet needs for assistance with symptoms, most commonly joint pain (29%), fatigue (25%), weight gain (23%), and difficulty with memory (21%). Overall levels of depressive (M=2.45) and anxiety (M=4.89) symptoms were low, and elevated depressive and anxiety symptoms were reported by 3% and 18% of the sample, respectively. Number of symptoms and anxiety were unrelated to any demographic, disease or treatment variables. Depressive symptoms and unmet needs were related younger age ($p < .05$) and depressive symptoms were further related to not having received radiotherapy ($p < .05$). Number of symptoms experienced and unmet needs were moderately related to both depressive (all $r > 0.49$, $p < .001$) and anxiety symptoms (all $r > 0.31$, $p < .01$).

Conclusion:

Among long term BC survivors symptoms are common, while unmet need and symptoms of anxiety and depression are more modest. However, both symptoms and unmet need are associated with symptoms of depression and anxiety and represent a potentially missed opportunity for improving outcomes among BC survivors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-13

Title: Enhancing compliance with national nutrition recommendations in breast cancer survivors. Experience in an underprivileged community

Zelek L, Festa A, Bodere C and Morello S. Ac'Santé93, Bobigny, France and Avicenne Hospital, AP-HP, Bobigny, France.

Body: **BACKGROUND:** To enhance compliance with national nutrition recommendations in breast cancer survivors (BCS), a 3-year program granted by the Regional Health Authority began in 2013 in an area (Seine-Saint-Denis, SSD) which is among the poorest in France.

PATIENTS AND METHODS: Ac'Santé 93 is a non-profit organization whose aim is to provide supportive care, health education and individualized assistance to patients and families, and to facilitate timely access to quality medical and psychosocial care. Vulnerability was evaluated using an 11-item standardized score (EPICES) previously investigated by French Health Examination Centers. This score is more strongly related to health status than the administrative classification of poverty (Sass, Sante Publique 2006). Vulnerability was defined by a score >30 and considered as severe when >40. Given the high level of poverty in the area and the incidence of financial difficulties in cancer survivors, a particular attention was paid to comparing the costs of different foods in order to promote affordable dietary changes. Between March 2013 and December 2015, 109 BCS were enrolled in a 3-month education program including 3 sessions of a professionally led support group (with dietitians and social workers). **RESULTS:** Ten BCS were lost for follow-up before the end of the program; 54 BCS attended all the sessions. Mean age was 52. Median vulnerability score was 51.8 (0-93.48) and 59% of patients had a score >30. Dietary intakes were assessed at baseline, and 1 and 6 mos. after the last session. At 1 mo. 65% BCS had knowledge of healthy dietary choices and 63% were ready to translate it into practice. Of note, 47% BCS decided to enroll in a tailored 1-year physical activity program or planned to do it, although it was not the aim of the study. At 6 mos. 52% of BCS still had knowledge of healthy diet and 49% of turned it into practice. However, only 12% were still practicing physical activity. Barriers were reported in 67% BCS and included asthenia or other treatment side effects (40%), anxiety or depression (27%), reluctance of relatives (20%), social isolation (20%) or cost (18%). Semi-directive interviews revealed unexpected benefits from this program such as empowerment, socialization or improvement of body image.

CONCLUSION: A short-term dietary intervention is feasible in vulnerable BCS living in an underserved area and improves adherence to higher quality diet in a meaningful number of patients. In spite of the attention paid to the affordability of dietary modifications, numerous barriers still exist in this population, the main one being treatment related side effects, including fatigue. Furthermore, compliance to a tailored physical activity program spontaneously decreases over time.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-14

Title: Factors associated with multidisciplinary care in the management of early stage breast cancer

Quyyumi F, Accordino MK K, Buono DL L, Neugut AI I, Hillyer GC C, Wright JD D and Hershman DL L. Columbia University Medical Center, New York, NY.

Body: Introduction: In patients with early stage breast cancer (BC) treated with curative intent, multidisciplinary teams (MDT) have emerged as a way to involve a wide range of specialists and encourage effective communication to formulate an optimal treatment strategy for patients. We sought to evaluate the frequency and predictors of MDT evaluation in patients with BC.

Methods: We used the Surveillance, Epidemiology, and End Results (SEER)-Medicare linked dataset to evaluate patients diagnosed with stages I and II breast cancer who underwent primary surgery from 2002-2007 and were followed through 2012. We evaluated claims for outpatient visits and characterized the treating physician as a surgeon, radiation oncologist (RO) or medical oncologist (MO). We defined MDT as having seen a physician in each of the three specialties within 12 months of diagnosis. We used multivariable logistic regression to evaluate factors associated with MDT.

Results: A total of 35,484 stage I and II breast cancer patients were included in the analysis. Within the first year, 77.5% visited a medical oncologist, 57.8% visited a radiation oncologist, and 47% of women were seen by all 3 specialists. Prior to surgery, 4.9% of patients were seen by all 3 physicians, with 14.8% seen by a MO and 16.4% seen by a RO in addition to the surgeon. Evaluation by a MDT was more frequent in women who had a lumpectomy vs mastectomy (57.1% vs 28.4%, $p < 0.0001$), Caucasian race as opposed to black and Hispanic (47.4% vs 42.1% vs 37.4%, $p < 0.0001$), those that lived in an urban setting versus rural (48.1% vs 36.25%, $p < 0.0001$), and those that were married versus unmarried (50.8% vs 43.1%, $p < 0.0001$). As age increased, the number of patients who saw all three physicians decreased. As socioeconomic status improved, more patients saw all three physicians. In a multivariate model, evaluation by a MDT was higher in patients with Stage II disease (OR [95% CI] = 1.10 [1.04-1.18]), diagnosed in 2006-2007 (as compared to 2002-2005) (OR = 1.73 [1.63-1.85]), and those who received chemotherapy (OR = 1.51 [1.39-1.64]) and was less likely for older women (OR = 0.77 [0.71-0.84]), those who underwent mastectomy (OR = 0.73 [0.68-0.78]), and those in the lowest socioeconomic quintile (OR = 0.88 [0.80-0.97]). Of those seen by all 3 physicians in the first year, 20.4%, 10.1%, 6.1%, and 3.9% were seen by all 3 specialists in years 2, 3, 4 and 5 respectively. Only 2.2% of patients saw all three specialists all five years.

Conclusions: Early stage breast cancer patients are evaluated by a medical oncologist, surgeon and radiation oncologist less than 50% of the time in the first year after diagnosis. Prior to surgery, where decision making may be most important, only 5% of patients were evaluated by all three specialties. Further research is needed to determine if MDT improves quality of care delivered, treatment adherence, patient satisfaction or breast cancer survival.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-15

Title: Longitudinal patterns of postdiagnosis weight changes according to adjuvant systemic treatments in Korean breast cancer survivors

Lee JW, Min Y, Chung IY, Son BH and Ahn SH. Asan Medical Center, Seoul, Republic of Korea and Gachon University, Incheon, Republic of Korea.

Body: The aims of this study were to investigate patterns of postdiagnosis weight changes from diagnosis to within 5 years since diagnosis and determine the association of adjuvant systemic treatments with weight gain or loss in Korean breast cancer survivors.

According to the types of adjuvant systemic therapy, 14540 patients whose body weight data could be retrieved from electronic medical record (EMR) system from January 2004 to December 2013 were divided into (1) no adjuvant therapy group (Gr 1, n = 1209), (2) endocrine therapy alone group (Gr 2, n = 5469), (3) chemotherapy alone group (Gr 3, n = 3122), (4) chemotherapy followed by endocrine therapy group (n = 4505), and (5) no available data group (n = 235). In this study, 9800 patients (Gr 1, 2, and 3) were included and their 5 years of follow up BMI data were analyzed. The numbers of follow up patients whose BMI data were available at 12th, 24th, 36th, 48th, or 60th month since diagnosis were 4908 (4908/9800, 50%), 4765 (4765/9800, 49%), 4745 (4745/9800, 48%), 4191 (4191/9800, 43%), and 4109 (4109/9800, 42%), respectively.

Paired comparisons among mean values of follow up body weights within each adjuvant group showed different longitudinal changing patterns: no statistically significant weight gains or losses in the Gr 1; significant weight loss only at 12 month in the Gr 2 (weight at 12th month - weight at diagnosis, mean \pm SD = -0.15 ± 2.9 kg, paired t-test, $p=0.01$); and significant weight losses consistently at all follow up months with the maximal weight loss at 24th month in the Gr 3 (weight at 24th month - weight at diagnosis, mean \pm SD = -0.78 ± 3.5 kg, paired t-test, $p < 0.01$). Comparisons of estimated body weights at each follow up month between the adjuvant groups (Gr 2 vs. Gr 1 and Gr 3 vs. Gr 1), which were calculated after adjusting two covariates such as initial weight and age at diagnosis, endocrine therapy was not associated with weight gain or loss throughout 5 years since diagnosis (ANCOVA, $p > 0.05$ at all follow up months), but chemotherapy was related to weight loss significantly emerged from 24th to 48th month since diagnosis (ANCOVA; $p > 0.05$, < 0.001 , 0.016 , 0.001 and > 0.05 at 12th, 24th, 36th, 48th, and 60th month, respectively).

Endocrine therapy showed no association with postdiagnosis weight changes, whereas chemotherapy was temporarily associated with postdiagnosis weight loss in Korean breast cancer survivors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-16

Title: Arthralgia-associated aging perceptions predict adherence to aromatase inhibitors among women with breast cancer

Mao JJ J, Chambless DL L, Chen J, Bao T and Brier MJ J. Memorial Sloan-Kettering Cancer Center, New York, NY and University of Pennsylvania, Philadelphia, PA.

Body: Background: Aromatase inhibitors (AIs) are associated with reduced risk of breast cancer recurrence, yet many women discontinue their treatment prematurely, often due to arthralgia. Empirically, breast cancer survivors who experience AI-associated arthralgia often report that they have aged quickly over a short period of time. **Objective:** We aimed to determine whether survivors with a heightened sense of aging due to arthralgia were more likely to non-adhere to their AI regimen. **Methods:** We conducted a prospective cohort study in an urban academic cancer center among post-menopausal women with hormone receptor positive breast cancer who were within the first two years of their aromatase inhibitor therapy. Perceptions of aging due to arthralgia were measured by the previously validated Penn Arthralgia Aging Scale. Non-adherence was defined as interrupting treatment or discontinuing the AI before the prescribed treatment length was over. Trained raters abstracted adherence data from medical charts. We performed Cox proportional hazards regression to evaluate the relationship between perceptions of aging due to arthralgia and time to non-adherence while adjusting for potential confounders. **Results:** Among 509 participants, most were White (81.2%) and had at least some college education (77.9%). The majority had been prescribed anastrozole (88.0%), followed by letrozole (9.0%), and exemestane (3.0%). During the follow up period, 144 (28.3%) did not adhere to the AIs as originally prescribed. In univariate analysis, women with a heightened sense of aging due to arthralgia were at more than twice the risk of non-adherence compared to women with low levels of aging perceptions (Hazard Ratio [HR], 2.20; 95% CI, 1.50 – 3.21; $p < 0.001$). After adjusting for arthralgia pain severity, depression, and AI type, aging perceptions remained a statistically significant predictor of adherence (HR, 1.71; 95% CI, 1.10-2.67; $p = 0.02$). Depression status also uniquely predicted non-adherence risk (HR, 1.63; 95% CI, 1.03 – 2.59; $p = 0.04$). Arthralgia pain severity, which predicted adherence in univariate analysis, was not a significant predictor in the final model (HR, 1.21; 95% CI, 0.84 – 1.75; $p = .30$). **Conclusions:** Breast cancer survivors on AIs who have a heightened sense of aging due to arthralgia are at greater risk of non-adhering to their AI regimen. Interventions are needed to help survivors reduce arthralgia and develop adaptive appraisals of their AI experience to achieve optimal adherence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-17

Title: Simplifying survivorship surveillance

Pories SE E, Myers LE E and Dusenbery KL L. Hoffman Breast Center Mount Auburn Hospital, Cambridge, MA.

Body: Breast cancer survivors represent 4 of 10 female cancer survivors in the United States, comprising an estimated 3.1 million women living with breast cancer in the US. Addressing survivors' unique post-treatment needs is critical to providing quality health care. In fact, Survivorship Care Plans are now required for all breast cancer patients by the National Accreditation Program for Breast Centers. To ensure that we meet recommended guidelines and provide comprehensive survivorship care, we have created a computerized treatment summary and developed a unique simple assessment tool to be incorporated at the patient's survivorship visit. The goal is to increase awareness of short and long term side effects post treatment and develop a plan to address the patient's needs. The visits are carried out by the nurse practitioners and average 60 minutes. Data from the first 20 patients in our new program show that most are age 40-49 (30%), 25% are age 60-69, 20% age 50-59, 20% age 70-79 and 5% age 30-39. The majority of patients (65%) were treated with breast conserving surgery and sentinel node biopsy. Most women who had a mastectomy also had reconstruction (83%). The majority (95%) had lymph nodes removed as part of their cancer surgery and 30% report a sensation of tightness or swelling on the side where lymph nodes were resected. The majority (90%) are receiving hormonal therapy and 40% also had chemotherapy. A majority (75%) reported side effects from hormonal therapy such as hot flashes, joint pain, or vaginal dryness. 15% of patients reported concerns with sexual function or sexual activity. Over a third of patients (35%) felt anxious, nervous, or on edge, 25% of patients reported that they had little interest or pleasure in doing things, 15% felt down, depressed or hopeless. Memory problems were reported by 30% of patients, 25% complained of difficulties multitasking or paying attention and 10% reported their thinking seemed slow. Over half of the patients (55%) reported problems with insomnia, 30% complained that fatigue interfered with their usual activities, 15% had persistent fatigue, and 15% reported excessive sleepiness. 25% complained of ongoing discomfort related to their cancer treatment but only 5% felt the pain interfered with their usual activities. There were no reports of difficulties with activities of daily living. However, 25% had concerns about their body image and appearance since cancer treatment. Most patients (90%) report eating a well-balanced diet and 65% engage in regular physical activity yet 45% of patients are concerned about their weight. Only 5% are smokers, 40% have up to 3 alcoholic beverages a week, 20% have 4-7 drinks a week, 10% drink more than 7 alcoholic beverages a week, and 25% do not drink alcohol at all. Only 50 percent had a recent pap smear, 45% had a recent colonoscopy, 60% had a recent bone density, and 80% see an ophthalmologist regularly. Most patients (60%) did not need referrals for other services, but 5% were sent for mental health services, 10% for consultation with the plastic surgeon, 10% for consultation regarding sexual functioning, 5% for physical therapy, and 5% for social work intervention. We have had an overwhelmingly positive response from patients to the survivorship visits and will continue to expand this useful and effective program.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-13-17

Title: The feasibility of nurse intervention to improve and maintain the BMI of breast cancer survivors over 25 to appropriate levels

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Body: Background: Some studies have shown that women with breast cancer who have a BMI value gain of more than 2.0 kg/m² after diagnosis have an elevated risk of dying of breast cancer, and likewise breast cancer patients who gained 5 or more kilogramshad a higher mortality rate than those who maintained their weight. Therefore it can be said that weight control is important for women with breast cancer.

In addition to the survival benefit, preliminary research suggests that exercise intervention for weight control may also be effective for enhancing the QOL of cancer survivors.

Purpose: We designed an exercise intervention study to assess the feasibility of the intervention in maintaining standard weight and also the effect of the exercise in breast cancer patients.

Patients and methods: Breast cancer patients aged 20 to 75 years, stage I to IIIA, with no metastases, who had completed chemotherapy, with BMI values greater than 25 kg/m² are eligible for participation. The intervention will be to have the patients do aerobic exercise 150 minutes per week, and assess the BMI and health-related QOL using the care note questionnaire, at baseline, 6 months, and 12 months later for each participant. We are also planning dietary interventions for the patients. We will calculate the actual calorie consumption of each patient and give dietary advice for optimal calorie intake. Patients will be required to record their exercise in a formatted note so we can check the exercise adherence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-14-01

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-14-02

Title: Triple negative breast cancer - Adjuvant chemotherapy use and survival outcomes in Stage IA disease

Patel AN N, Shi R, Peddi P and Burton GV V. Feist Weiller Cancer Center, Louisiana State University Health Sciences Center, Shreveport, LA.

Body: Background: The potential benefit of adjuvant chemotherapy in patients with Stage IA triple negative breast cancer (TNBC) has not been defined. In general, patients with T1a and T1b lesions have not been included in adjuvant chemotherapy trials and the inclusion of T1c tumors has been limited. In this study using National Cancer Data Base (NCDB) we investigated the actual use of adjuvant chemotherapy in Stage IA TNBC patients relative to tumor size (T1a, T1b, T1c) and report their survival outcomes.

Patients and Methods: Using NCDB we evaluated a cohort of 13,065 women with TNBC diagnosed between 2010-2012 who had American Joint Committee on Cancer Stage IA (node-negative with pathological T1a, T1b or T1c) tumors. Overall survival (OS) was the primary outcome variable. Based on the tumor size, patients were stratified on receipt of adjuvant chemotherapy or not. Patients were also stratified according to receipt of adjuvant radiation, radiation with boost, or none. Other adjusted variables included: age, race, Charlson comorbidity index, payer status, income, education, distance traveled, treating facility, and treatment delays. Multivariate Cox regression was employed to analyze the effect of adjuvant chemotherapy on overall survival.

Results: The mean patient age for the entire cohort was 59.2 years (range 22-90 years), 55.8 years for the chemotherapy group, and 67.8 years for the non-chemotherapy group. There were 1275 T1a, 3197 T1b, and 7729 T1c patients. Tumor size was a very strong predictor of survival. Compared to T1a tumors, HR for death was 1.43 (95% CI: 0.86 –2.37) for T1b tumors and 3.00 (95% CI: 1.86 – 4.83) for T1c tumors. Out of all T1a, T1b, and T1c tumors in this cohort, 48.1 %, 72.6%, and 89.3% of patients received adjuvant chemotherapy respectively. A hazard ratio (HR) of death was 0.42 (95% CI: 0.31 – 0.57) for all patients who received chemotherapy compared to non-chemotherapy group. 4-year OS by tumor size and chemotherapy usage is listed in the table indicating an absolute increase of OS with adjuvant chemotherapy employment. HR for death was 0.90 (CI: 0.62 – 1.31) with use of radiation only and 0.67 (95% CI: 0.53 – 0.85) with use of radiation with boost when compared to no radiation therapy.

4-year OS (in percentage) with and without adjuvant chemotherapy use for node-negative T1a, T1b, and T1c TNBC

Tumor size	No Chemotherapy	Chemotherapy	P Value
T1a	93.78 %	98.36 %	0.146
T1b	91.91 %	97.10 %	<0.0001
T1c	80.62 %	94.41 %	<0.0001

Conclusion: NCDB indicated that the majority of patients with Stage IA TNBC received adjuvant chemotherapy, including 48% of patients with T1a lesions. Our data analysis demonstrated a statistically significant 4-year OS benefit in patients with T1b and T1c tumors who received adjuvant chemotherapy compared to those who did not. The survival benefit of adjuvant chemotherapy in patients with T1a tumors, however, did not reach statistical significance. Prospective randomized trials could define the potential benefits of adjuvant chemotherapy in patients with Stage IA TNBC, particularly for those with T1a and T1b tumors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-14-03

Title: Adjuvant dose dense doxorubicin-cyclophosphamide (ddAC) or docetaxel-AC (TAC) for high-risk breast cancer: First results of the randomized MATADOR trial (BOOG-2004-04)

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Body: Background: Anthracycline-based adjuvant chemotherapy has substantially improved breast cancer survival. Both the addition of taxanes as well as using a dose dense treatment schedule can further ameliorate outcome, but inter-individual differences exist. Here we present the efficacy and toxicity of dose dense scheduled doxorubicin/cyclophosphamide (ddAC) versus docetaxel/doxorubicin/cyclophosphamide (TAC), which is, to our knowledge, the first direct comparison of these treatment regimens.

Methods: In this Dutch, multicenter phase III trial (ISRCTN61893718), patients with pT1-3, pN0-3, M0 breast cancer were randomized between six cycles of either A₆₀C₆₀₀ every 2 weeks or T₇₅A₅₀C₅₀₀ every 3 weeks. All patients received pegfilgrastim. Patients were evaluated for recurrence-free survival (RFS) and overall survival (OS). Survival was compared in a Cox regression analysis and adjusted for known prognostic factors. These factors include age, type of surgery, tumor size, histological grade, ER/PR status, HER2 status, and lymph node status. Adverse events were reported according to the common toxicity criteria (CTCAE version 3.0).

Results: Between 2004 and 2012, 664 patients were enrolled of whom 531 (80%) had node positive disease. At a median follow up of 5 years, OS was 92% in the ddAC treated subgroup and 93% in the TAC treated subgroup (adjusted hazard ratio [HR] 0.75, 95% confidence interval [CI] 0.42-1.34, intention to treat population). Forty-two breast-cancer specific deaths were equally divided over both treatment arms. Similarly, no significant difference in RFS was observed between both treatment groups (adjusted HR 0.85, 95% CI 0.55-1.32). Molecular subtypes were defined by St. Gallen criteria: 548 patients (83%) had estrogen receptor positive disease and 102 patients (15%) triple negative disease. No heterogeneity regarding treatment efficacy was observed in these subtypes. In particular, there was no survival benefit for ddAC or TAC in the triple negative subtype. Both treatment regimens were well tolerated. Whereas anemia was more frequent in ddAC treated patients (19% vs 4.7%; p<0.001), peripheral neuropathy occurred more frequently in TAC treated patients (4.6% vs 14.4%; p<0.001). The frequency of febrile neutropenia was not significantly different between the treatment arms (11% vs 12.5%; n.s.). Six patients developed congestive heart failure: 2 ddAC treated patients, 4 TAC treated patients. One ddAC treated patient and one TAC treated patient were diagnosed with acute myeloid leukemia after study treatment; another patient in the ddAC treatment group developed myelodysplastic syndrome.

Conclusions: At a median follow up of 5 years no significant survival differences were observed between adjuvant ddAC and TAC, in all patients and in molecular subgroups, including triple negative. Our findings are in line with the Oxford overview, which reported no significant differences between taxane-based chemotherapy and more, non-taxane based chemotherapy given in a dose dense schedule. ddAC could be a reasonable alternative for patients with a contra-indication for TAC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-14-04

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-14-05

Title: Capecitabine in early breast cancer: A meta-analysis of randomized controlled trials

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Body: Background

Capecitabine is an effective therapy for metastatic breast cancer. Its role in early breast cancer is uncertain due to conflicting data from randomized controlled trials (RCTs).

Methods

PubMed and major conference proceedings were searched to identify RCTs comparing standard chemotherapy (defined as cyclophosphamide/methotrexate/5-fluorouracil, anthracycline-based regimens or anthracycline/taxane combinations) with or without capecitabine in the neo-adjuvant or adjuvant setting. Hazard ratios (HR) for disease-free (DFS) and overall survival (OS), as well as odds ratios (ORs) for safety and tolerability were extracted or calculated and pooled in a meta-analysis. Subgroup analysis compared triple negative breast cancer (TNBC) to non-TNBC and whether capecitabine was given in addition to or in place of standard chemotherapy. Meta-regression was used to explore the influence of TNBC on OS.

Results

Eight studies comprising 9302 patients were included. In unselected patients, capecitabine did not influence DFS (HR 0.99, $p=0.93$) or OS (HR 0.90, $p=0.36$). There was a significant difference in DFS when capecitabine was given in addition to, compared to in place of standard treatment (HR 0.92 vs. 1.62, interaction $p=0.002$). Addition of capecitabine to standard chemotherapy was associated with significantly improved DFS in TNBC vs non-TNBC (HR 0.72 vs. 1.01, interaction $p=0.02$). Meta-regression confirmed this association with OS ($R=-0.967$, $p=0.007$). Capecitabine increased Grade 3/4 diarrhea (OR 2.33, $p<0.001$) and hand foot syndrome (OR 8.08, $p<0.001$), and resulted in more frequent treatment discontinuation (OR 3.80, $p<0.001$).

Conclusion

Adding capecitabine to standard chemotherapy appears to improve DFS and OS in TNBC, but increases adverse events in keeping with its known toxicity profile. Consideration of this treatment is warranted, especially in high-risk patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-14-06

Title: The effect of adjuvant chemotherapy in a large consecutive series of ER-pos HER-2 negative early breast cancers

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Body: Background: The last EBCTCG-overview reported, in general, a significant better breast cancer outcome if adjuvant chemotherapy was added to surgery and endocrine therapy in ER-pos breast cancers, but the precise indication for adjuvant chemotherapy in this population remains controversial. We study the effect of adjuvant chemotherapy on breast cancer outcome in consecutive patients with an ER-pos HER-2 negative breast cancer treated with adjuvant antihormonal therapy.

Methods: Data were collected prospectively from consecutive patients with non-metastatic breast cancer that were primary operated between 2000 and 2012 at the University Hospitals Leuven (Belgium). A Propensity Score (PS) weighted analysis was performed to estimate the average treatment effect (ATE). The primary endpoint was recurrence free interval (RFI). Secondary endpoints were distant recurrence free interval (D-RFI) and breast cancer specific survival (BCSS). Covariates used to generate the propensity score and to include in the PS weighted analysis were age at diagnosis, body mass index, tumor size, grade, pN, lymph vessel invasion, PR, and radiotherapy. Cause-specific hazard models were fitted, using death not from breast cancer as competing risk. Treatment heterogeneity was examined by evaluating interactions of each covariate with adjuvant chemotherapy, using the Bonferroni-Holm method to correct for multiple testing.

Results: In the total cohort of 5609 patients, 4282 had a hormone sensitive HER-2 neg breast cancer and 4121 (96.2%) of these received adjuvant antihormonal therapy. Adjuvant chemotherapy was given in 1179/4121 patients (29%). Median follow-up was 8.5 years. Due to very strong differences between patients with and without adjuvant chemotherapy, a restricted PS weighted analysis was used according to a recent recommendation in the statistical literature. This analysis is based on 1750 patients with a PS between 0.1 and 0.9. In this group, 807 (46%) received adjuvant chemotherapy, 211 (12%) observed an event for RFI, 167 (10%) for D-RFI, and 108 (6%) for BCSS. Adjuvant chemotherapy was associated with better prognosis: the adjusted cumulative incidence of recurrence within 5 years was 9.7% without and 5.3% with adjuvant chemotherapy. The adjusted hazard ratio for RFI was 0.50 (95% CI 0.33-0.74). There were no strong interactions with adjuvant chemotherapy. Results for D-RFI and BCSS were similar.

Conclusion: Based on PS analysis to reduce confounding and chemotherapy indication bias, we observed clear benefit from adjuvant chemotherapy in ER-pos HER-2 negative breast cancers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-14-07

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-14-08

Title: Adjuvant and neoadjuvant chemotherapy for elderly patients (≥ 70 years) with early high-risk breast cancer: A retrospective analysis of 116 patients

Servent V, Tresch E, Vuagnat P and Bonnetterre J. Centre Oscar Lambret, Lille, France.

Body: Background: adjuvant or neoadjuvant chemotherapy in elderly patients (pts) is currently considered as a toxic treatment and reserved for fit women and high-risk breast cancer; few guidelines are available in this age group. The aim of this study was to evaluate the tolerability of chemotherapy in this patient population. **Patients and Methods:** We performed a retrospective analysis of the use of adjuvant and neo adjuvant chemotherapy in our Cancer Center in two groups of patients (≥ 70 y-75y and >75 y) with early high-risk breast cancer. Between 2009 and 2014, 116 consecutive breast cancer patients (90pts:70-75y and 26pts:75>y: range70-84) received adjuvant or neoadjuvant chemotherapy; all patients had optimal surgery. The chemotherapy regimens were 3 FEC100 (epirubicine 100mg/m² + 5-fluorouracil and cyclophosphamide 500mg/m²) + 3 docetaxel100mg/m² or 4 TC(docetaxel 75mg/m² + cyclophosphamide 600mg/m²). In case of HER2 positive breast cancer (IHC3+ or Sish+), the patients received trastuzumab for one year. The treatment is considered non optimal when there was a decrease in the number of courses or a dose reduction. However Docetaxel75mg/m² after 3 FEC100 was considered acceptable as well as weekly administration. A yearly follow-up was performed after the end of the chemotherapy. The association between clinical data and age group (70-75) or (>75) was analyzed with Khi-2 test or Fisher test. **Results** Pts > 75 y had more ≥ 3 co-morbidities: (38.5% vs 18.9% (p= 0.04), had more frequently triple negative breast (TN) breast cancer (53.8% vs 20% (p=0.001), inflammatory breast cancer (19.2% vs 3.3%) (p=0.014). Pts >75 y had less frequently RH+ breast cancer (23.1% vs 61.1% (p=0.001); there was no significant differences for N+ between the two groups. The >75 y pts received more frequently growth factors: 57.7% vs 35.6% (p=0.043); they had more often comprehensive geriatric assessment: 42.3% vs 11.1% (p<0.001) and were more often hospitalized (38.5% vs12.2% (p=0.002). There was no differences between the two age groups for Her2 status, SBR histoprognostic grading, grade ≥ 3 toxicity, 1year and persistent toxicity. One patient died in the group 70y-75y because of sepsis. There was no differences between the age groups in the rate of decrease of chemotherapy dosing. There was a statistical association between co-morbidities and grade ≥ 3 toxicity (p=0.004), grade ≥ 3 toxicity and hospitalization rate (p<0.001), grade ≥ 3 toxicity and chemotherapy dosing (p<0.001). Conversely no association was found between chemotherapy dosing and 1 year health status. One year Trastuzumab was well tolerated in both groups. **Conclusions:** Adjuvant or neo adjuvant chemotherapy for elderly high-risk breast cancer patients is feasible. The >75 years old patients, those with ≥ 3 co morbidities are especially frail, at risk of toxicity, hospitalization, inadequate chemotherapy. These results questioned about the best tools for survey and the value of geriatric intervention along the treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-14-09

Title: Luminal A breast cancer: Is it really a “good prognosis” disease? Preliminary results of the GIM-13 - AMBRA study

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Body: BACKGROUND

Luminal breast cancer is a highly heterogeneous disease comprising different histologies, gene-expression profiles and mutational patterns, with different clinical behavior and responses rate to systemic treatments. It has been shown that IHC determined ER, PgR, and HER2/neu status largely describe the subgroups when genetic profiling data is unavailable. There is strong evidence from some studies that chemotherapy does not add benefit when compared with endocrine therapy alone in defined subgroups resembling and including luminal A disease.

Despite adjuvant endocrine therapy and chemotherapy treatment for patients at high risk of relapse, both early and late relapses still occur, a fact that highlights the unmet medical needs of these patients.

PATIENTS AND METHODS

The AMBRA Study is a longitudinal cohort study, aiming at describing the choice of first and subsequent lines of treatment in HER2-ve ABC pts. Data of 30 consecutive pts per centre at first disease relapse in the years 2012-2015 and treated with chemotherapy (CHT) for the metastatic disease are collected. For the purpose of this analysis, we identified, basing on IHC expression of ER and PgR, all the Luminal A cases in the whole study population registered in the AMBRA Study, with the aim of describing the type of adjuvant therapy, the sites of relapse and the treatment offered as 1st-line therapy.

RESULTS

At the moment of the present preliminary analysis, 747 out of the planned 1200 pts have been enrolled in the main study. 234 cases of Luminal A tumours (31.3%) have been identified. Median age at diagnosis of the primary tumour was 50 years (42-76). Ductal carcinoma was the most frequent histology (68.3%). 148 pts (63.2%) have received CHT as part of their adjuvant treatment. The most used regimens were anthracycline-based therapies (77, 52%), and anthracyclines+taxanes (41, 27.7%). Median Disease Free Interval was 133 months (1-390). At the moment of 1st relapse, a biopsy was done in 134 cases (57.3%) to obtain new biology information: a concordance between primary biology and metastatic one was defined in 84 cases (66.7%), 40 cases lost PgR expression, becoming Luminal B tumors, and 10 cases lost both receptors (TNBC). Main sites of relapse were bone (58.9%), viscera in 50.4% and soft tissue (12.8%). As 1st-line treatment, 162 (69.2%) pts received CHT; the most frequently used CHT were the combination of Paclitaxel+Bevacizumab (36, 22.2%). Single agent CHT was chosen in 66 cases (40.7%), mainly Paclitaxel (33, 20.3%). Among pts treated with CHT as 1st-line, 81 pts (74.3%) received a 2nd-line CHT, as single agent (52, 64.2%; Capecitabine 15, 18.5% Nab-paclitaxel 10, 12.3%).

CONCLUSION

These preliminary results of the GIM 13 - AMBRA Study show that Luminal A tumours have a quite long DFI, but relapse at visceral sites in half of the cases, often requiring the use of a CHT regimen. Despite guide lines recommend the use of single agent sequential CHT, a vast majority of the pts are still treated with a combination regimen.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-14-10

Title: Weekly doxorubicin and daily oral cyclophosphamide followed by nab-paclitaxel for adjuvant therapy of high-risk localized breast cancer

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Body: **BACKGROUND:** The addition of taxanes to anthracycline-based adjuvant chemotherapy has improved disease free survival (DFS) in women with high-risk early-stage breast cancer. Many studies have sought to optimize the dose intensity and density of these agents to produce improvements in outcome and tolerability. The purpose of this study was to assess the use of metronomic doxorubicin plus daily oral cyclophosphamide (AC) for 12 weeks followed by nab-paclitaxel (nP) for 12 weeks in this population. Those patients with Her2 positive disease were also given adjuvant trastuzumab.

METHODS: A non-randomized phase II clinical trial was designed to (1) test the DFS at 2 years compared to historical controls, (2) assess dose intensity delivered, (3) assess use of nP in the adjuvant setting, and (4) evaluate toxicities associated with the regimen. Overall survival (OS) was a secondary outcome. The dosing of A was 24mg/m² IV qweek and C was 60mg/m² oral daily; nP, 100mg/m² IV qweek.

RESULTS: Sixty patients were enrolled on the study with a median follow-up of 6 years and a median age of 50 (range 30-69). 58% of patients had node positive disease. Receptor categories included hormone receptor positive (ER positive or PR positive) and HER2 negative (n=24; 40%); ER negative, PR negative, and HER2 negative (triple negative; n=19; 32%); or HER2 positive (n=17; 28%). DFS at 2 years was 93% (1 death, 3 recurrence) and at 6 years was 82%, comparable to historical controls. OS at 2 years and 6 years was 98% and 88%, respectively.

Disease-free survival and overall survival at 2 and 6 years

	2 year		6 year	
	DFS %	OS %	DFS %	OS %
All patients	93	98	82	88
ER+ or PR+, HER2-	92	100	79	79
Triple negative	89	95	79	89
HER2+	100	100	88	100

Mean dose intensity was greater than 90% for AC and 88% for nP. Treatment was well-tolerated with the most common grade ≥ 3 toxicity being neutropenia and a 2% incidence of febrile neutropenia.

CONCLUSIONS: Patients achieved similar DFS to that seen in historical controls with similar rates of adverse events. Since nP dosing was 100 mg/m², even with 88% dose intensity, the delivered taxane dose is greater than standard weekly paclitaxel. Notably disease control was particularly impressive in the triple negative subtype, which has been shown to benefit from nP over standard paclitaxel in the neoadjuvant setting in the GeparSepto (GBG 69) trial. Metronomic AC followed by nP is a safe, effective option for delivery of adjuvant chemotherapy for high-risk patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-14-11

Title: An estimation of the population survival benefit of first-line chemotherapy and immunotherapy for breast cancer

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Body: Purpose: Randomized clinical trials describe the benefit of chemo-and immunotherapy for specific breast cancer patients with selected patient and disease characteristics. However, variability in practice occurs despite evidence-based guidelines [1]. The overall survival benefit for the whole population of breast cancer patients in Australia, if evidence-based guidelines for chemo-and immunotherapy were implemented, is unknown. Our study's purpose was to estimate the overall population survival benefit of routinely using evidence-based practice.

Methods and Materials: Decision trees with evidence-based indications for chemotherapy have been previously defined [2]. Each branch corresponds to a specific cohort who have, or do not have, defined indications for chemotherapy and/or immunotherapy. Chemo -and immunotherapy benefit was defined as the absolute incremental benefit of either chemotherapy and/or immunotherapy over no chemo- and/or immunotherapy for radical and palliative indications. Multiple electronic citation databases were systematically queried, including Medline and the Cochrane Library. In cases where there were multiple sources of the same level of evidence, hierarchical meta-analysis was performed. The benefits of chemo- and immunotherapy were estimated for 1, 5, 10-year survival. To assess the robustness of our estimates, sensitivity analyses were performed.

Results: The estimated 1-year, 5-year and 10-year absolute population-based overall survival benefits of optimally utilized chemo- and immunotherapy for breast cancer in Australia are 1.0% (95% CI, 0.9%-1.2%), 4.4% (95% CI, 4.3%-4.6%) and 5.2% (%-%), respectively. They are summarized in the Table 1.

Estimation of Population Survival Benefit for First Line Chemo- and Immuno Therapy

Breast Cancer	Proportion of all cancer in Australia	1 year survival benefit (Sensitivity range)	5 year survival benefit (Sensitivity range)	10 year survival benefit (Sensitivity range)
Stage I-II	10.0%	0.6% (0.6%-0.7%)	4.8% (4.6%-5.0%)	6.9% (6.7%- 7.2%)
Stage III	1.6%	3.0% (3.0%-3.1%)	6.1% (5.8%-6.3%)	0%
Stage IV	0.5%	5.3% (5.1%-5.5%)	4.9% (4.7%-5.1%)	0%
Whole Breast Cancer population	12.1%	1.0% (0.9%-1.2)	4.4% (4.3%-4.6%)	5.2% (5.0%-5.4%)

Conclusion: Chemo- and immunotherapy agents improves overall survival in breast cancer at 1-, 5- and 10-years. Chemo-and immunotherapy provides a modest survival benefit to this patient population in Australia when it is used in accordance with guideline recommendations. These outcomes may allow comparison of treatment outcomes in a jurisdiction against what would be considered optimal based on evidence.

1. Fong, A., et al., *A comparison of systemic breast cancer therapy utilization in Canada (British Columbia), Scotland (Dundee), and Australia (Western Australia) with models of "optimal" therapy.* Breast, 2012. **21**(4): p. 562-9.
2. Ng, W., *Estimating the optimal chemotherapy utilisation rate as an evidence-based benchmark in cancers of the breast, upper gastrointestinal tract, gynaecological tract, head and neck, kidney, bladder, thyroid and unknown primary.*, in University of NSW, Faculty of Medicine. 2010, UNSW: Sydney.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-14-12

Title: Long-term outcome of HER2-normal early stage breast cancer (ESBC) patients (Pts) treated with docetaxel-cyclophosphamide (TC) chemotherapy (CTx): Mature results of a single-institution experience

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Body: BACKGROUND

Anthracycline(A)-containing regimens (AReg) became an established standard (neo)adj CTx for ESBC following fairly consistent demonstration of a modest superiority over older anti-metabolite/alkylating CTx. However, substantial translational data and a recently presented pooled analysis [Blum, 2016] suggest that this superiority could be largely driven by greater benefits in specific ESBC subgroups, i.e. HER2-altered BrCa (due to co-amplification of topoisomerase 2 and HER2), and triple-negative BrCa (TNBC). A are cardiotoxic (including late onset of cardiomyopathic congestive heart failure) and potentially leukaemogenic. In late 2006, following the results of the first USONC randomized clinical trial that showed superior outcomes of the non-AReg TC (docetaxel/cyclo-phosphamide) over AC, we established a routine, uniform policy of TC for all Pts receiving (neo)adj CTx for HER2-normal ESBC. We report the mature follow up of this single-institution unselected experience.

METHODS

We performed a retrospective outcome analysis of all Pts who received at least 1 cycle of (neo)adj TC (docetaxel 75 mg/m² + cyclophosphamide 600 mg/m² IV every 3 weeks) at our Department for HER2-normal ESBC and with at least 5 years of follow up (FU). Pts were identified by systematic analysis of the dataset of the Oncology Pharmacy Unit. Information on tumour characteristics [e.g. axillary lymph nodes (N) metastases, hormonal receptors (HR) and HER2 status] and Pts FU were retrieved and collected into an *ad hoc* designed database. Pts with node-positive (N+) ESBC received TC×6 cycles, and Pts with high-risk node-negative (N-) [e.g. primary tumour (T) >2 cm, or HRneg, or T >3 cm] ESBC received TC×4 cycles. Pts received adjuvant hormone therapy and radiotherapy as per standard of care. From 2008 on, many lower risk HR+/N- Pts were not given CTx due to OncotypeDx availability.

RESULTS

Between September 2006 and December 2015, 810 female HER2-normal ESBC Pts were treated with (neo)adj TC. In the final outcome analysis we included 464 Pts treated before June 2011 thus having a minimum FU of 5 years. Pts characteristics are: median age 53 yrs (range 30-77), N- 246 (53%), N+ 218 (47%), hormone receptors positive (HR+) 391 (84%), TNBC 73 (16%). The database was locked as of June 1st 2016. Median FU from first cycle of TC is 7.5 yrs (range 5.3-10). 63 BrCa-specific relapse events (defined as time to local, regional or distant recurrence, invasive contralateral breast cancer, excluding non-breast second primaries) have been observed, accounting for an overall Relapse-Free Survival (RFS) rate of 86.4%. 42 deaths have occurred, 36 (86%) due to BrCa, accounting for an Overall Survival (OS) rate of 91%. RFS and OS rates for the different Pts subgroups are reported in Table 1

Table 1 - Outcome parameters

	RFS (%)	OS (%)
All Pts	86	91
HR+/N-	93	96
HR+/N+	81	90
TN/N-	91	91
TN/N+	58	58

CONCLUSIONS

These mature data with long FU suggest that the outcome for a large cohort of unselected Pts with HER2-normal HR+ ESBC (regardless of nodal status) and for TN/N- ESBrCa treated with nonAReg TC is excellent. However, N+TN ESBrCa in this setting remains a significant clinical challenge.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-02

Title: Phase 1b/2 study to evaluate eribulin mesylate in combination with pembrolizumab in patients with metastatic triple-negative breast cancer

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Body: Background: Eribulin, a non-taxane microtubule inhibitor, is approved for the treatment of (1) patients (pts) with advanced or metastatic breast cancer (MBC) who have received ≥ 1 [European Union] or ≥ 2 [United States] prior chemotherapy regimens for metastatic disease, including an anthracycline and taxane in either the adjuvant or metastatic setting and (2) pts with unresectable or metastatic liposarcoma who have received a prior anthracycline regimen. Pembrolizumab is a human programmed death receptor-1 (PD-1)-blocking antibody approved for the treatment of (1) pts with unresectable or metastatic melanoma and disease progression following ipilimumab and, if *BRAF*^{V600}-mutation positive, a BRAF inhibitor and (2) pts with advanced non-small cell lung cancer with tumors expressing PD-L1. Pembrolizumab is also being evaluated for the treatment of metastatic triple-negative breast cancer (mTNBC) and other tumor types. Here, we report an interim analysis from an open-label, single-arm, multicenter, phase 1b/2 study to evaluate the safety and efficacy of the eribulin and pembrolizumab combination in pts with mTNBC previously treated in the metastatic setting.

Methods: A total of 95 pts (aged ≥ 18 yrs, ECOG PS 0 or 1) with mTNBC previously treated with ≤ 2 prior lines of chemotherapy for metastatic disease will be enrolled. Phase 1b included a safety run-in cohort in which ≥ 6 pts received intravenous (IV) eribulin mesylate 1.4 mg/m² on day (d) 1 and d8 and IV pembrolizumab 200 mg on d1 of a 21-d cycle. Dose-limiting toxicity (DLT) of the combination regimen were assessed in the first cycle to determine the recommended phase 2 dose (RP2D). In phase 2, patients were enrolled in 2 cohorts according to receipt of prior chemotherapy in the metastatic setting (0 vs 1–2 prior lines). Primary endpoints were determination of safety and tolerability (phase 1b) and evaluation of objective response rate (phase 2); secondary endpoints included evaluation of progression-free survival, overall survival, and duration of response.

Results: We report an interim analysis of the first 39 enrolled pts (n=7, phase 1b; n=32, phase 2). No DLTs were observed in phase 1b. The RP2D was defined as eribulin 1.4 mg/m² on d1 and d8 and pembrolizumab 200 mg on d1 of a 21-d cycle. As of data cut-off (May 16, 2016), the most frequent adverse events (AEs; all grades) were fatigue (69%), nausea (51%), alopecia (36%), neutropenia (36%), and peripheral neuropathy (28%). The most frequent AEs of grade 3 or 4 were neutropenia (31%) and fatigue (8%). Serious AEs (all nonfatal) occurred in 36% of pts and AEs leading to dose adjustment were observed in 56% of pts. Objective responses, including a complete response, by investigator assessment were observed during this interim analysis, and will be presented at the meeting.

Conclusions: The combination of eribulin with pembrolizumab represents a novel treatment regimen in pts with mTNBC. AEs observed with the combination were comparable to those observed with either treatment as monotherapy.

Clinical Trials.gov: **NCT02513472**.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-03

Title: *nab*-paclitaxel + carboplatin or gemcitabine vs gemcitabine/carboplatin as first-line treatment for patients with triple-negative metastatic breast cancer: Results from the randomized phase 2 portion of the tnAcity trial

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Body: Background: *nab*-Paclitaxel (*nab*-P)–containing regimens have demonstrated efficacy and safety in triple-negative breast cancer (TNBC). In the absence of a standard of care for metastatic (m) TNBC, tnAcity evaluated the efficacy and safety of 3 common chemotherapy combination regimens: *nab*-P + carboplatin (*nab*-P/C) or gemcitabine (*nab*-P/G) vs G/C as first-line treatment (Tx) for patients (pts) with mTNBC based on a ranking algorithm of efficacy and safety. Results of the phase 2 portion are reported here.

Methods: Pts with pathologically confirmed mTNBC, no prior cytotoxic chemotherapy for metastatic disease, and no brain metastases were enrolled. Pts received (1:1:1) *nab*-P 125 mg/m² + C AUC 2, *nab*-P 125 mg/m² + G 1000 mg/m², or G 1000 mg/m² + C AUC 2, all given on d 1 and 8 every 3 weeks. The phase 2 primary endpoint was investigator-assessed progression-free survival (PFS); secondary endpoints included overall response rate (ORR), overall survival (OS), percentage of pts initiating cycle 6 receiving doublet therapy, and safety.

Results: 191 pts were included in the phase 2 portion. Median age was 55, 53, and 59 years in the *nab*-P/C, *nab*-P/G, and G/C groups, respectively. Overall, 98% of pts had an ECOG PS of 0 - 1, 83% were white, and 48% were treated in North America. Most pts (81%) had relapsed disease. Median Tx duration was 25, 18, and 20 weeks, and 47%, 33%, and 52% of pts had ≥ 1 dose reduction for both agents in the *nab*-P/C, *nab*-P/G, and G/C groups, respectively. Key efficacy and safety results are summarized in the table.

Table 1

	<i>nab</i> -P/C	<i>nab</i> -P/G	G/C
Efficacy	n = 64	n = 61	n = 66
PFS, median, mo	7.4 ^a	5.4 ^b	6.0
ORR, %	71.9	37.7	43.9
OS, median, mo	16.4	11.9	13.7
Pts initiating cycle 6 with a doublet, %	64.1	55.7	50.0
Safety	n = 64	n = 60	n = 64
Myelosuppression-related events, % ^c	53.1	41.7	67.2
Pts discontinuing all Tx due to AE, %	18.8	20.0	21.9
Serious TEAEs, %	29.7	36.7	37.5
Grade ≥ 3 AEs, %			
Neutropenia	40.6	33.3	48.4
Anemia	7.8	8.3	15.6
Thrombocytopenia	4.7	3.3	14.1

Peripheral neuropathy	4.7	5.0	1.6
Deaths due to TEAE, n (%)	1 (2)	2 (3)	2 (3)

^a P = 0.03 vs nab-P/G; P = 0.02 vs G/C; ^b P = 0.85 vs G/C; ^c Including grade \geq 3 neutropenia, thrombocytopenia, anemia, bleeding, febrile neutropenia, or red blood cell/platelet transfusion.

PFS was significantly longer with *nab*-P/C vs either *nab*-P/G or G/C (median, 7.4 vs 5.4 mo, $P = 0.03$ and 7.4 vs 6.0 mo, $P = 0.02$). Overall, 179 pts discontinued Tx; of these, 55% discontinued due to progression and 16% due to adverse events (AEs). Grade \geq 3 AEs in \geq 10% of pts were mainly hematologic. The rank sum of the algorithm for 5 key weighted efficacy and safety endpoints favored *nab*-P/C.

Conclusions: In this phase 2 study, *nab*-P/C demonstrated a statistically significant longer PFS and a better risk/benefit profile vs *nab*-P/G or G/C as first-line Tx in pts with mTNBC. These data demonstrate *nab*-P/C efficacy in mTNBC and address a relevant question around a *nab*-P/C chemotherapy backbone for this difficult-to-treat tumor type. Further studies are warranted in the context of immunotherapies and targeted agents. NCT01881230.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-04

Title: The PROCEED trial KCSG BR11-01 phase III multicenter randomized open-label study of irinotecan plus capecitabine versus capecitabine in patients previously treated with anthracycline and taxane for HER2 negative metastatic breast cancer

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Body: Purpose

We investigated whether the combination of irinotecan plus capecitabine improved progression free survival (PFS) compared with capecitabine alone in patients with HER2 negative MBC previously exposed to anthracyclines and taxanes.

Patients and methods

A total of 211 patients were randomly assigned to irinotecan (80mg/m² on D1 and D8) and capecitabine (1,000mg/m² bid on D1 to D14) or capecitabine alone (1,250mg/m² bid on D1 to D14) every 3 weeks. The primary objective was PFS; secondary objectives included overall response rate, overall survival and safety.

Results

Both arms were well balanced in terms of age, hormone receptor status, visceral involvement, and number of previous treatment

Table 1. Patient characteristics

	IX(N=111)	X(N=100)	p-value
Age (yr, median, range)	50 (29-73)	49 (30-80)	0.47
ECOG			0.80
0	25 (22.5%)	22 (22%)	
1	85 (76.6%)	76 (76%)	
2	1 (0.9%)	2 (2%)	
Pre-menopause	28 (25.2%)	29 (29%)	0.64
Post-menopause	83 (74.8%)	71 (71%)	
ER/PgR			0.16
positive	60 (54.1%)	64 (64%)	
negative	51 (45.9%)	36 (36%)	
Adjuvant Chemotherapy	86 (77.5%)	72 (72%)	0.43
Adjuvant Endocrine	46 (41.4%)	39 (39%)	0.78
Visceral meta			1.0
yes	64 (57.7%)	58 (58%)	
no	47 (42.3%)	42 (42%)	
Previous Chemotherapy			0.40
0	12 (10.8%)	12 (12%)	
1	60 (54.1%)	46 (46%)	
2	32 (28.8%)	34 (34%)	
3	5 (4.5%)	8 (8%)	
4	2 (1.8%)	0	

IX, irinotecan plus capecitabine; X, capecitabine.

. There was no significant difference in PFS between the combination and capecitabine monotherapy arm (median, 6.6 vs. 5.3 months; HR=0.87; 95% CI, 0.65 to 1.16; P=0.33). In patients with triple negative breast cancer (N=87), the combination treatment significantly improved PFS (median, 4.8 vs. 2.8 months; HR=0.59 ; 95% CI, 0.37 to 0.94; P=0.03). Overall response rate was higher in the combination arm though it did not reach statistical significance (42.7% vs. 29.6%, P=0.06). Overall survival did not differ between two groups (median, 2.2 vs. 1.7 years; P=0.47). Grade 3 or 4 neutropenia occurred in 39.6% in the combination arm and 10% in the monotherapy arm. Hand-foot syndrome (\geq grade 2) was more common in the monotherapy arm (23.0% vs. 12.6%).

Conclusions

Irinotecan plus capecitabine did not demonstrate superior clinical activity in heavily treated HER2 negative MBC patients. The role of adding irinotecan to capecitabine in triple negative breast cancer remains to be elucidated.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-05

Title: Randomized phase II study evaluating different schedules of nab-paclitaxel in metastatic breast cancer (MBC): Results of the SNAP study

Gennari A, Sun Z, Hasler-Strub U, Colleoni M, Kennedy J, von Moos R, Cortes J, Vidal M, Hennessy B, Walshe J, Amillano Parraga K, Morales Murrillo S, Pagani O, Barbeaux A, Borstnar S, Rabaglio M, Maibach R, Regan MM M and Jerusalem G. International Breast Cancer Study Group (Bern, Switzerland), Cancer Trials Ireland (Dublin, Ireland), and SOLTI (Barcelona, Spain).

Body: Background

Longer chemotherapy (CT) duration is associated with a significant improvement in progression-free survival (PFS) and a moderate, but significant improvement in overall survival (OS) in MBC patients (pts). Prolonged CT administration, however, must be weighed against the side effects of continuous CT delivery. The SNAP trial was designed to improve the tolerability of prolonged CT by studying alternative treatment schedules.

Methods

The SNAP trial enrolled 258 women from April 2013 to Aug 2015. Eligibility criteria included HER2- MBC, no prior CT for advanced disease, measurable and/or non-measurable disease.

All eligible pts were randomized to one of three arms. Pts received the same induction chemotherapy consisting of 3 cycles of nab-Paclitaxel given days 1,8,15 Q28, followed by one of the three maintenance therapy schedules. Originally, the dose of the induction chemotherapy was 150 mg/m², but this was reduced to 125 mg/m² following the first safety review of 48 treated pts. The three schedules of nab-Paclitaxel used as maintenance therapy were (Arm A) nab-Paclitaxel 150 mg/m² d 1,15 Q28; (Arm B) nab-Paclitaxel 100 mg/m² d 1,8,15 Q28; (Arm C) nab-Paclitaxel 75 mg/m² d 1,8,15,22 Q28.

The primary objective is to evaluate the efficacy of three nab-Paclitaxel regimens as measured by progression-free survival (PFS), using the historical reference of PFS (based on AVADO study) of docetaxel for first-line treatment of metastatic breast cancer. Each of the three regimens is compared to the historic 7-month median PFS to determine whether any of the three regimens are worthy of further investigation. Secondary endpoints include tolerability, feasibility, response rate, OS and QoL.

Results

Two-hundred-fifty-eight pts have been randomised and 255 are available for primary endpoint evaluation. At 18.2 months' median follow-up, 182 PFS events and 85 deaths have been observed. Median PFS was 7.9 months (90%CI 6.8-8.4) in Arm A, 9.0 months (90%CI 8.1-10.9) in Arm B and 8.5 (90%CI 6.7-9.5) in Arm C. PFS in Arm B was significantly longer than the historic PFS of first-line docetaxel (one-sided log-rank p=0.03).

As expected, neurotoxicity was the most frequent adverse event. In the induction phase, grade \geq 2 sensory neuropathy was reported in 14.8% of pts at the starting dose of 150 mg/m² and 7.5% at the starting dose of 125 mg/m²; grade \geq 3 sensory neuropathy occurred in 2.5% and 0% of the pts, respectively. In the maintenance phase, grade \geq 2 sensory neuropathy was reported in 37.9% of pts in Arm A, 36.1% in Arm B and 31.2% in Arm C; grade \geq 3 sensory neuropathy occurred in 9.1%, 5.6% and 6.6% of the pts, respectively.

199 pts started the maintenance phase. The median number of maintenance cycles was 3, 4, and 5, respectively. Stopping maintenance for reasons other than objective progression occurred in 41%, 58%, and 53%, respectively.

Conclusion

The SNAP trial indicates that alternative maintenance chemotherapy schedules with reduced doses after a short term induction phase at conventional doses are feasible and significantly more active than the historical PFS of docetaxel in the first line treatment of advanced breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-06

Title: Impact of post-progression therapy on overall survival (OS) in the IMELDA randomized phase III trial evaluating the addition of capecitabine (CAP) to maintenance bevacizumab (BEV) for HER2-negative metastatic breast cancer (mBC)

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Body: BACKGROUND The open-label randomized phase III IMELDA trial demonstrated that adding CAP to maintenance BEV until disease progression after initial BEV + docetaxel (DOC) provides statistically significant and clinically meaningful improvements in both progression-free survival (PFS [primary endpoint]; hazard ratio [HR] 0.38, 95% CI 0.27–0.55; log-rank $p < 0.001$; median 4.3 vs 11.9 months with BEV vs BEV + CAP, respectively) and OS (HR 0.43, 95% CI 0.26–0.69; median 23.7 vs 39.0 months, respectively) [Gligorov, Lancet Oncol 2014]. The type and extent of post-progression therapy were not collected prospectively; to address questions from the oncology community and provide further insight into the observed OS benefit with CAP, we collected these data retrospectively in the post-IMELDA study.

METHODS In IMELDA, patients (pts) with HER2-negative measurable mBC and no prior chemotherapy for mBC received 3-6 cycles of BEV + DOC. Pts with a response or stable disease were randomized to BEV alone or BEV + CAP (BEV 15 mg/kg d1 q3w; CAP 1000 mg/m² bid d1-14 q3w) until disease progression, unacceptable toxicity, or consent withdrawal. OS from randomization was a secondary endpoint. In post-IMELDA, data until study closure were collected retrospectively from available records of pts randomized in IMELDA. Ethics, consent, and feasibility reasons prevented some sites participating in post-IMELDA.

RESULTS Post-IMELDA data were available from 118 (64%) of the 185 randomized pts. Of these, all but 8 had received further systemic anticancer therapy and 92 of 118 (78%) had received chemotherapy. Post-progression treatment exposure and efficacy are shown below. In Cox regression models stratified by further anticancer therapy after stopping study therapy, HRs were 0.30 (95% CI 0.19–0.48) for PFS and 0.50 (95% CI 0.30–0.82) for OS. Sensitivity analyses using two extremes of imputation (assuming all pts with missing follow-up data did vs did not receive further anticancer therapy) supported OS findings.

Parameter	BEV (n=59)	BEV + CAP (n=59)
Any further systemic anticancer therapy, n (%)	56 (95)	54 (92)
Chemotherapy	49 (83)	43 (73)
CAP	34 (58)	7 (12)
Non-CAP-containing	43 (73)	41 (69)
Targeted therapy/immunotherapy	12 (20)	3 (5)
BEV	8 (14)	1 (2)
Endocrine therapy	24 (41)	31 (53)
Other	11 (19)	10 (17)
Median PFS, months (95% CI)	4.3 (2.7–7.4)	15.0 (9.9–19.3)
Subgroup with further anticancer therapy	4.3 (3.5–7.4)	14.1 (9.9–19.3)
Median OS, months (95% CI)	22.3 (12.1–29.8)	36.5 (27.6–NE)
Subgroup with further anticancer therapy	22.9 (16.5–31.7)	36.5 (24.0–NE)

NE=not estimable

CONCLUSIONS Post-IMELDA is limited by retrospective collection of data from only two-thirds of randomized pts and only until the date of study closure (thus potentially underestimating crossover to CAP). Nevertheless, these analyses suggest that the OS benefit from adding CAP to maintenance BEV in the IMELDA trial was robust despite extensive post-progression therapy in both treatment arms and crossover to CAP in more than half of pts randomized to maintenance BEV alone. Ongoing and future mBC trials should include prospective collection of information on subsequent therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-07

Title: First and further line choices of treatment for HER2-VE metastatic breast cancer (MBC) according to adjuvant treatment and biological subtype. Preliminary results of the observational "GIM-13 – AMBRA" Italian study

Mustacchi G, Cazzaniga ME E, Biganzoli L, De Laurentiis M, Del Mastro L, De Placido S, Donadio M, Garrone O, Giordano M, Latini L, Livi L, Marchetti P, Michelotti A, Montemurro F, Natoli C, Pronzato P, Riccardi F and Turletti A. Università di Trieste; ASST Monza; Ospedale Santo Stefano Oncologia Medica; INT - Fondazione G. Pascale; I.R.C.C.S. A.O.U. San Martino - I.S.T.; A.O.U. Federico II; A.O.U. Citta della Salute e della Scienza Oncologia Medica Senologica; A.O. S. Croce e Carle di Cuneo - P.O. Carle Oncologia; ASST Lariana - Ospedale S. Anna U.O. di Oncologia Medica; Ospedale di Macerata U.O. Oncologia; A.O.U. Careggi Radioterapia; Azienda Ospedaliera Sant' Andrea U.O.C. Oncologia; Azienda Ospedaliera Pisana U.O. Oncologia Medica I; Fondazione del Piemonte per l'Oncologia - Istituto di Ricovero e Cura a Carattere Scientifico (I.R.C.C.S.) Direzione di Oncologia Clinica; Policlinico 'SS. Annunziata' Clinica Oncologica; Azienda Ospedaliera 'A. Cardarelli' (AORN) U.O.C. Oncologia and Presidio Ospedaliero Martini - ASL TO1 D.H. Oncologia.

Body:

Frequency of CHT regimens according to biological subtypes and Setting of treatment

Luminal Tumours		Setting			
Regimen	Adj (%)	1st-line (%)	2nd-line (%)	3rd-line (%)	
anthra-based (w/o Taxanes)	44.2	7.1	5.1	9.9	
Anthra-Taxanes	30.5	2.6	0.5	0	
CMF-like	17.5	0.6	1.0	2.1	
Taxane mono/combo*	6.3	19.3	14.5	21.5	
Paclitaxel + Bevacizumab	0	20.2	4.3	3.7	
Platinum-based	0	3.6	1.7	0.8	
Capecitabine +/- Vinorelbine	0	19.1	22.2	17.4	
Eribuline	0	0	2.3	12.4	
TNBC		Setting			
Regimen	Adj (%)	1st-line (%)	2nd-line (%)	3rd-line (%)	
Anthra-based (w/o Taxanes)	25.7	3.1	5.1	16.2	
Anthra-Taxanes	36.1	1.1	0	0	
CMF-like	8.2	2.1	5.1	0	
Taxane mono/combo*	6.3	18.4	17.8	10.8	
Paclitaxel + Bevacizumab	0	24.7	1.7	0	
Platinum-based	0	20.6	22.4	2.7	
Capecitabine +/- Vinorelbine	0	25.7	20.6	10.8	
Eribuline	0	0	3.4	29.7	

**Irrespectively of biological subtype, nab-paclitaxel was used in 3.6%, 15.3% and 16.4% of cases in 1st, 2nd and 3rd line, respectively

BACKGROUND

Breast Cancer prognosis improved in the last years due both to early diagnosis and Adj treatments. The choice of CHT regimens

should consider previous Adj treatments, pattern of relapse and biological subtype. There are few information on the treatment of MBC in the clinical practice outside controlled trials, last study has been published 15 yrs ago in Italy.

PATIENTS AND METHODS

The GIM13-AMBRA Study is a multicenter longitudinal cohort study, describing the choice of first and subsequent lines of treatment for MBC in HER2-ve pts. We are collecting data of 30 consecutive pts from 42 Italian Centres who developed the first relapse in the years 2012-2015 and were treated with CHT, (+/- previous endocrine treatments (HT)) for MBC. One of the secondary aims is to evaluate the Time to Treatment Change (TTC) (time between treatment start and its change for any reason) as surrogate endpoint for Time To Progression outside clinical trials. The present report is focused on the choice of treatments in any line and TTC, according to biological subtypes. .

RESULTS

For the present analysis, 683 pts are evaluable. Pts with Luminal A and B tumours received CHT and HT in 65.3% (Adjuvant), 21.7% (1st line), 7.1% (2nd line) and 7% (3rd line) of the cases. The most used regimens according to tumour subtype and line of treatment are shown in Table 1.

Median TTC from 1st to 2nd line was 8.1 and 17.9 months in TNBC and Luminal tumours, respectively, whereas TTC from 2nd to 3rd line was 3.1 and 12.9 months, respectively.

CONCLUSION

Preliminary results of the AMBRA-GIM13 Italian observational study confirm that in most cases treatment for MBC is strongly related to the type of the Adj regimen, being the use of anthracyclines marginal in MBC, whereas taxanes are widely used in any line. In 1st line the most used regimens are Taxane and Bevacizumab or Capecitabine/Vinorelbine combinations. The last one remains the most used CHT in 2nd and 3rd lines. No difference have been observed according to biological subtype, except for Platinum-based regimens in TNBC. HT alone remains the preferred choice in 1st and 2nd line in Luminal cases. TTC seems to be a reliable surrogate for PFS in the “real world” practice . CHT still plays a crucial role in the treatment of MBC HER2-ve pts.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-08

Title: A phase 2 study of eribulin as early-line treatment for HER2- MBC: Evaluation of efficacy, toxicity, and patient-reported outcomes

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Body: Background:

Patient-reported outcomes (PROs) independently and directly describe patient (pt) treatment experiences, complement clinician reports, and are increasingly included in drug development. Eribulin (E) has activity in metastatic breast cancer (MBC) previously treated with ≥ 2 regimens. Limited data are available on the activity of E in earlier lines. This phase II single arm study evaluated the activity, safety, and PROs of E in an earlier line setting for HER2- MBC.

Methods: Eligible pts had HER2- MBC, 0-1 prior lines of chemotherapy, and enrolled into one of two parallel cohorts: HR+, TNBC. E was dosed 1.4 mg/m² weekly, 2 wk on/1 wk off; 1 cycle = 3 wks. The primary objective was overall response rate (ORR); additional objectives included progression-free survival (PFS) and safety. Correlative PRO endpoints included description and comparison of toxicity evaluation by PROs vs provider-report for degree of concordance or divergence for each class of toxicity, and description of QOL over time. Neuropathy was evaluated by the FACT-Neurotoxicity subscale, as well as exploration of predictive host polymorphisms. PRO instruments (PRO-CTCAE, FACT-B and FACT-NTX) were administered electronically at baseline, cycles 1-3, then every other cycle.

Results: 83 pts enrolled: 45 HR+ and 38 TNBC. Median age was 56 (range: 34-87). 55.4% were first line, and 66% had received previous taxane in the adjuvant or metastatic setting. ORR for all pts was 25.3% (90% CI: 18%-34%); 35.6% in HR+ (90% CI: 24%-49%) and 13.2% in TNBC (90% CI: 5%-26%). mPFS estimate was 5.8 months (mo) for all pts, HR+ 6.2 mo (90% CI: 5.9-8.7) and TNBC 4.0 mo (90% CI: 3.5-4.8). Related toxicities occurring in $\geq 20\%$ of pts were: fatigue (61.4% overall, 32.5% G1, 27.7% G2, 1.2% G3), alopecia (51.8% overall, 16.9% G1, 32.5% G2), nausea (39.8% overall; 26.5% G1, 12.0% G2, 1.2% G3), peripheral sensory neuropathy (36.1% overall; 18.1% G1, 13.3% G2, 4.8% G3), decreased neutrophil count (32.5% overall; 3.6% G1; 4.8% G2, 13.3% G3, 10.8% G4), peripheral motor neuropathy (22.9% overall, 13.3% G1, 6.0% G2, 3.6% G3), and mucositis (20.5% overall, 16.9% G1, 2.4% G2, 1.2% G3). FACT-B Breast Cancer Concern (BCC) and Trial Outcome Index (TOI) scores were stable during therapy. The greatest mean improvement in summed BCC (mean change 3.3) and TOI (mean change 7.9) compared to baseline occurred at cycle 9 in pts with stable disease. Changes from baseline in the NTX and TOI of FACT-NTX worsened during the first 7 cycles of therapy (mean changes NTX 5.3, TOI 4.8). Pharmacogenomic analysis exploring host polymorphisms and development of neuropathy is ongoing.

Conclusions: PROs are indispensable in the evaluation of anti-cancer agents in clinical trials. In this phase II trial, E demonstrated activity and safety in early lines of therapy for HER2- MBC. Additionally, improved QOL metrics were observed among pts experiencing stable disease. CTCAE rates of severe neuropathy were low, however PRO testing by FACT-NTX highlights progressive worsening of neurotoxicity. In contrast to classic CTCAE criteria producing aggregate estimates of toxicity, this phase II trial shows longitudinal collection of PROs provides meaningful, complementary, and dynamic clinical information.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-09

Title: Evaluation of growth modulation index as a marker of benefit for consecutive lines of treatment for metastatic breast cancer

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Body: Background: Sequencing of single-agent chemotherapy (CT) is the current standard of treatment for endocrine-resistant metastatic breast cancer (MBC). However, shrinking benefit for consecutive lines of CT and lack of data from randomized trials may question the real value of advanced lines of treatment. Growth modulation index (GMI), the ratio of progression free survival (PFS) with PFS of previous line, has been reported as a marker of treatment benefit when equal to or above 1.3. This value is similar to the clinical benefit threshold proposed by ESMO and ASCO for non-curative CT. The aim of this work was to determine GMI change across the successive lines of CT for MBC.

Methods: We retrospectively analyzed all MBC patients currently on treatment at our center. GMI was determined for each CT line and for each patient and a cut-off value of 1.3 was chosen as a marker of treatment benefit. Chi-squared test and Spearman's Rho were used for proportion comparison and correlations. Kaplan-Meier curves and log-rank test were performed for survival analysis.

Results: 128 ABC patients were included; median age: 57; ECOG 0-1: 92.2%; visceral disease: 46%; bone-only disease: 46.9%; TNBC: 5.5%, HER2+: 23.5%, ER+HER2-: 71.1%; 50% more than 2 lines of CT. GMI and PFS values are shown in Table 1. GMI values were significantly associated with PFS values in all lines ($p < 0.001$ for 2nd, 4th and 5th lines; $p \leq 0.01$ for 3rd and 6th). GMI was neither significantly different between CT lines nor significantly correlated with GMI of previous line. No consistent association of GMI values with age, performance status, tumor subtype or visceral disease were found.

Table 1. GMI and PFS values for each line of CT

CT line	n	GMI Median (range)	GMI \geq 1.3	PFS Median (95%CI)
2nd	63	0.88 (0.03-27)	36.5%	10.4 months (7.2-13.2)
3rd	39	0.62 (0.05-7.5)	23.1%	4.9 months (4.1-5.7)
4th	28	0.83 (0.05-12.6)	21.4%	5.3 months (1.1-9.4)
5th	20	0.68 (0.05-9.1)	30.0%	4.7 months (1.5-7.9)
6th	14	0.80 (0.25-22.8)	42.9%	4.5 months (2.0-7.1)

Conclusions: GMI does not significantly change along successive CT lines for MBC. Our results, showing a constant rate of 20-40% of patients with $GMI \geq 1.3$ up to the sixth line of CT, suggest a benefit within the range of magnitude established by ASCO and ESMO framework for cancer care value. Although potential biases of this work warrant further evaluation with a cohort design, GMI, together with toxicity data and absolute gains of PFS, may be used as an additional tool to establish the real value of late lines of CT for MBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-10

Title: Eribulin mesylate (eribulin) showed inhibitory effects on epithelial-mesenchymal transition (EMT) in tumors of metastatic breast cancer patients. -First preliminary report of a prospective study-

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Body: Background: EMT is thought to contribute to metastasis in patients with breast cancer, leading to their poor prognosis. Pivotal phase III trials have demonstrated that eribulin improved overall survival in patients with triple negative metastatic breast cancer (MBC). Preclinical studies have shown that eribulin suppressed EMT and this phenomenon could be one of reason for an improved prognosis of MBC patients treated with eribulin. However, there is no direct clinical data on the effect of eribulin treatment on EMT in tumors of MBC patients. We designed a prospective study to clarify if eribulin treatment suppresses EMT in tumors of MBC patients.

Patients and methods: Patients with recurrent or MBC were treated with eribulin (1.4 mg/m² intravenously on days 1 and 8 of a 21-day cycle). Dose reductions were allowed according to eribulin's prescribing information. Treatment continued until disease progress, unacceptable toxic effects, or discontinuation requests from patients or physicians. Breast cancer tissue samples were obtained from all patients before and on day 15±4 of 1st cycle of eribulin treatment. The quantitative analysis of mRNA levels of EMT markers (E-cadherin, cytokeletin18, cytokeletin19, N-cadherin, vimentin, ZEB1, Slug, Snail, and Twist) were carried out by qPCR. Primary outcome measure was to assess the change from baseline to day 15±4 in mRNA levels of EMT related markers in tumor tissue. This study was approved by the Ethic Committee of Fujita Health University.

Results: Eleven patients were enrolled. Median age of the patients was 63 years old (44-72). Of the 11 tumors, 6 were luminal B and 5 were triple negative (TN). Median number of prior chemotherapy regimen for recurrent or metastatic disease was 0 (0-3). Four patients were treated with dose reduced eribulin (1.1 mg/m²) and administration of eribulin on day 8 during 1st cycle of the treatment were skipped in 2 patients. To identify meaningful EMT markers, differences in expression levels of each EMT marker were investigated between TN and luminal B tumors. At baseline mRNA levels of N-cadherin and vimentin were higher in TN tumors than in luminal B tumors, 8.12±10.78 vs 1.02±0.68, 5.13±4.50 vs 0.88±0.47, respectively. After the treatment, a decrease of expression of N-cadherin and vimentin was more frequent in TN tumors (100% and 80%, respectively) than in luminal B tumors (33.3% and 16.7%, respectively). Frequency of a decrease of expression of ZEB1, Slug, Snail, and Twist in TN tumors and luminal B tumors were 80% (TN) vs 83.3% (luminal B), 80% vs 16.7%, 40% vs 66.7%, and 100% vs 50%, respectively.

Conclusions: This is the first prospective study to investigate the effect of eribulin treatment on expression of EMT markers in tumors of MBC patients. We demonstrated that eribulin treatment suppressed EMT in tumors. Our results suggested that eribulin showed antitumor effect by improving the tumor microenvironment. In our study, eribulin seems to have different effects on EMT pathway in individual cases. Our findings may provide a light to a scientific basis for solving underlying mechanisms for improvement of overall survival of patients with MBC treated with eribulin.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-11

Title: Eribulin should be a candidate strategy in combination with pertuzumab plus trastuzumab for taxane pretreated HER2 positive advanced breast cancer

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Body: Background: Pertuzumab (P) improves clinical outcome when combined with docetaxel and trastuzumab (T). The efficacy of continuing multiple anti-HER2 therapy including P and/or T after initial progression is unclear. Eribulin mesylate (ERI) is able to overcome taxane (TAX) resistance advanced breast cancer (ABC). The objective of this study is to investigate the efficacy and safety of ERI plus P and T for both TAX and T pretreated HER2-positive ABC. **Methods:** This is a single institute, open-label, single-arm, Phase II study with pharmacokinetics (PK) of ERI (UMIN000012375). The initial dose of P is 840 mg, followed by 420 mg q3w; the initial dose of T 8 mg/kg, followed by 6 mg/kg q3w; ERI is administered at 1.4 mg on Days 1 and 8 of each cycle, q3w. Dose reductions of ERI (to 1.1 and 0.7 mg/m²) were permitted to manage any toxicity (more than grade 3). Patients (Pts) must have previous treatment with both TAX and T. The primary endpoint is assessed overall response rate (ORR). Secondary endpoints include progression-free survival (PFS), overall survival (OS), safety, and PK of ERI. Left ventricular ejection function (LVEF) was evaluated before and end of this study. **Results:** Thirty Pts were enrolled. Median age at baseline was 57 years. Half of Pts had endocrine positive. All Pts were treated with TAX and T. Twenty-one Pts were treated with anthracycline-based treatment (70%). Median number of previous chemotherapy was 4 (2-5). Pts had multiple metastases, 40% with bone, 36.7% with lung, 20% with liver, and 10% with brain. Pts received a median number of 8 cycles of ERI (mean dose 1.2 ± 0.19 mg/sqm), 8 cycles of both P and T. Total number of 27 Pts needed to reduce dose of ERI because of adverse events (AEs) especially grade 3 neutropenia. The ORR (CR+PR) was 34.8% (95% CI 16.4-57.3, n=23) with median PFS of 42.6 weeks (95% CI 20.3-51.9, n=30). Clinical benefit rate (CR+PR+≥6 month SD) was 60.9% (95% CI 16.4-57.3%). T-DM1 pretreated affected poor outcome than the other factors (p=0.0011). The most common grade 3/4 AEs were neutropenia in 20 Pts (66.7%) without febrile neutropenia. Grade 1/2 AEs were fatigue in 24 Pts (80%), anorexia in 23 Pts (76.7%), anemia in 22 Pts (73.3%), diarrhea in 20 Pts (66.7%), peripheral neuropathy in 16 Pts (53.3%), and hand-foot syndrome in 12 Pts (43.3%). Baseline LVEF was 67%. One Pt had asymptomatic LVEF decrease (below an absolute value of 55%). Otherwise, there was no overall decrease in mean LVEF from baseline. Nine points (pre-dose, end of infusion, 0.5, 1, 2, 4, 24, 72, and 168 h after ERI) of PK analyses were evaluated in 6 Pts, and 3 point (pre-dose, end of infusion, and 168 h after ERI) in 10 Pts. PK parameters of ERI were as follows; Maximum plasma concentration (C_{max}) was 375.96 (257.6-531.8) ng/ml, terminal half-life was 36.807 (31.90-40.80) h, total clearance was 1.945 (1.15-3.15) L/h/m². C_{max} of ERI was not correlated with neutrophil count (R²=0.2338, n=16). **Conclusions:** The combination of ERI plus P and T was well tolerated; no new safety signals were observed. PK parameter of ERI were as same as previous reports when combined with both P and T. ERI might be a one of strategy in combination with P plus T for TAX pretreated HER2 positive ABC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-12

Title: Real-life activity of oral vinorelbine in metastatic breast cancer patients in the Unicancer ESME database

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Body: Background: In 2014, UNICANCER, composed of 18 French Comprehensive Cancer Centers, launched the Epidemiological Strategy and Medical Economics (ESME) program to investigate real-world data in oncology. Real-world data give the opportunity to assess the activity of specific products outside the framework of clinical trials. Oral vinorelbine (OV) is one of the therapeutic options available for metastatic breast cancer (mBC). Few data are available regarding its real clinical efficiency in current practice. We aimed at evaluating such activity within the ESME population.

Methods: The ESME-mBC database was built from information systems, treatment databases and patients' electronic files, with homogenous on-site collected information and high-level quality-control (Delaloge et al, Ann. Oncol 2016 (in press)). All patients having started a systemic treatment for mBC in a cancer center participating in the ESME program between 01-Jan-2008 and 31-Dec-2013 have been selected into the database.

For the purpose of the current analyses, data cut-off was July, 2015 and all patients who received OV at any time during the course of their disease were selected and analyzed. Primary end point was progression-free survival (PFS) from initiation of OV. Secondary end points were descriptive and prognostic analyses, and overall survival (OS).

Results: Among 13.853 patients recorded in the ESME-mBC database, 1402 received OV as a monotherapy or in combination (809 and 593 patients / 57.7% and 42.3% respectively). Most frequent combinations were with capecitabine (368 patients) and anti-HER2 therapy (165 patients). De-novo mBC was observed in 282 patients (20.1%) and 569 patients (40.6%) had only non-visceral metastases. At metastatic diagnosis, 221 patients (16.9%) had HER2-positive and 298 patients (22.9%) triple-negative tumors respectively. At OV initiation, median age was 59.0 [IC95%: 50-67] years. Endocrine therapy was given prior OV in 769 patients (54.9%). For PFS analysis, 1345 patients were evaluable. The following table summarizes PFS results according to the treatment patterns and the OV-line.

PFS results according to the treatment patterns and the OV-line

		OV-line			
		1st line	2nd line	3rd line	4th line and more
Overall population	N	320	414	313	270
Overall population	PFS (months, [IC95%])	4.7 [4.2-5.5]	3.3 [3.0-3.5]	2.9 [2.6-3.2]	2.3 [2.1-2.4]
OV monotherapy	N	118	223	207	202
OV monotherapy	PFS (months, [IC95%])	4.3 [3.1-5.3]	3.2 [2.9-3.5]	2.8 [2.5-3.2]	2.2 [2.0-2.4]
OV in combination with capecitabine	N	153	129	49	25
OV in combination with capecitabine	PFS (months, [IC95%])	5.1 [3.9-6.1]	3.5 [2.8-4.6]	3.0 [2.4-4.7]	2.0 [1.4-5.2]
OV in combination with anti-HER2	N	41	50	38	26
OV in combination with anti-HER2	PFS (months, [IC95%])	6.0 [4.1-7.9]	3.1 [2.4-3.8]	3.3 [2.4-6.0]	2.7 [1.9-3.7]

Following diagnosis of mBC, median OS was 38.2 months [IC95%: 36.1-40.0] in the cohort of patients who received OV at any time.

Conclusions: This study allows large scale assessment of real life benefit of OV over subsequent lines and shows that OV yields clinical benefit even in heavily pre-treated mBC patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-13

Title: Capecitabine in combination with bendamustine in pretreated women with HER2-negative metastatic breast cancer: Final PFS results of a phase II trial (AGMT MBC-6)

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Body: Background: Capecitabine is a well-established treatment option in HER2-negative advanced breast cancer (ABC) patients. Bendamustine is a generally well tolerated cytotoxic drug. Since bendamustine has already shown anticancer activity in ABC we evaluated the efficacy and tolerability of bendamustine in combination with capecitabine in pretreated patients with ABC. Here we present the final PFS results of this phase II trial.

Patients and methods: MBC-6 is a non-randomized, multicenter, open-label, single-arm phase II study in patients with HER2-negative ABC (ClinicalTrials.gov: NCT01891227). All patients were pretreated with anthracyclines and/or taxans and had measurable disease according to RECIST 1.1. Patients received 1000 mg/m² capecitabine twice daily on days 1 to 14 in combination with 80 mg/m² bendamustine on day 1 and 8 of a 3-week cycle for a maximum of 6 cycles. Afterwards capecitabine was continued as monotherapy. The primary endpoint was overall response rate (ORR). Secondary endpoints were progression-free survival (PFS), clinical benefit rate (CBR), safety and quality of life.

Results: From September 2013 to May 2015, 40 patients were recruited in eight Austrian centers. Median age was 60 years (range 29-77). Twenty-five percent of patients had triple-negative disease (TNBC) and 93% showed visceral involvement. Sixty-five percent had received prior chemotherapy in the (neo)adjuvant setting and 63% for ABC (43% one line, 15% two lines, 5% three lines). All patients with ER-positive disease had received prior endocrine therapy. At data cut-off on 06/08/16 overall 39 of 40 patients had discontinued treatment with a median PFS of 7.0 months (95% CI 4.6-9.5), 7.4 months in ER-positive and 4.0 months in triple negative disease (TNBC), respectively. Twelve patients (30%) experienced at least one drug related non-hematological AE \geq grade 3 during combination treatment and further 6 patients (15%) during capecitabine maintenance. Three grade 4 hematological AEs (neutropenia) were observed. One patient died as a result of restrictive cardiomyopathy, where a relationship to capecitabine cannot be excluded, but seems unlikely.

Conclusion: The combination of capecitabine and bendamustine shows promising efficacy and a moderate toxicity profile. Further evaluation of this drug combination is warranted.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-14

Title: Metronomic capecitabine in heavily pretreated metastatic triple negative breast cancer

El-Sadda WM M. Mansoura University, School of Medicine, Mansoura, Egypt.

Body: Background:

Triple negative breast cancer (TNBC) is defined histologically as an invasive carcinoma of the breast that lacks staining of ER, PgR and HER2/neu. Metastatic TNBC is an aggressive subtype of breast cancer marked by higher rate of visceral and CNS metastatic as well as poor survival. Metronomic chemotherapy regimens have shown efficacy in patients with metastatic breast cancer by enhancing the antiangiogenic mechanism. Capecitabine monotherapy is active against metastatic breast cancer and when used metronomically, the toxicity profile is low. The purpose of this study is to evaluate the metronomic capecitabine in patients with MTNBC in terms of response rate, toxicity profile, progression free survival and overall survival.

Patients and methods:

Forty patients with measurable metastatic/recurrent TNBC during the period from Jan 2012 to Jan 2014, patients had WHO PS <2, adequate renal, liver and bone marrow functions. Capecitabine administered orally in a low dose of 500 mg/m² twice daily and continued until progression or death. Response was assessed every two months, with any evidence of disease progression; patients were withdrawn from the study.

Results:

All patients were evaluated for response rate and toxicity. The median age was 50 years (range 36-59), median WHO PS was 1. Main metastatic sites were liver 40%, lung 30%, bone 40%, skin 10% and local recurrence 20%. Fifty percent of patients had one metastatic site, 40% had two metastatic sites and 10% had more than two metastatic sites. Partial response (PR) was observed in 12 patients (30%), stable disease (SD) in 20 patients (50%), and progressive disease in 8 patients (20%). Median time to progression was 5 months and median survival 9 months. Grade 2, 3 and 4 hematological toxicities and clinical side effects were uncommon. Hand and foot syndrome (G1) was experienced by 8 patients (20%).

Conclusion:

Metronomic capecitabine was effective and minimally toxic in heavily pretreated patients with metastatic TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-15

Title: Phase II study of navelbine plus carboplatin followed by oral navelbine in patients with metastatic/recurrent triple negative breast cancer previously treated with anthracycline and taxane

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Body: Background:

Navelbine (N) and carboplatin (C) are both active drugs in the treatment of breast cancer, N is a semisynthetic drug belongs to the vinca alkaloids, its oral form has proved to be effective as the iv form. Metastatic triple negative breast cancer (TNBC) is an aggressive subtype of breast cancer marked by higher rate of visceral and CNS metastases as well as poor survival. We investigated the efficacy and safety of IV navelbine plus carboplatin followed by oral navelbine in patients with metastatic TNBC in terms of response rate, toxicity profile, progression free survival (PFS) and overall survival (OS).

Patients and methods:

Between Dec 2011 and Dec 2013, forty patients with histologically proven measurable metastatic/recurrent TNBC were enrolled in the study. Patients had history of previous treatment with anthracyclines and taxanes. WHO PS <2, adequate renal, liver and bone marrow functions. Treatment consisted of navelbine 25mg/m² D1 & D8 (IV infusion over 6-10 min) and carboplatin AUC 5 D1 (IV infusion over 30 min), repeated every three weeks. Response was assessed every two cycles, patients showed an objective response (CR, PR or SD) had continued to 8 cycles followed by oral navelbine 60 mg/m² D1 & D8 for the first cycle then escalated to 80 mg/m² D1 & D8 every three weeks for six months.

Results:

All patients were evaluated for response, toxicities and survival. Median age was 43 years (range 36-56) and median WHO PS 0. Main metastatic sites were liver 40%, lung 30%, bone 60%, skin 10%, and local recurrence 20%. Fifty percent of patients had one metastatic site, 30% had two metastatic sites and 20% had more than two metastatic sites. The ORR after navelbine/carboplatin was 65% (CR 15%, PR 50%), SD in 25% and PD in 10%, while after oral navelbine, the ORR was 75% (CR 25%, PR 50%), SD 16.6 and PD 8.4%. The median PFS & OS were 12.3 & 19.8 months respectively. No G 3 & 4 toxicities were noted. G2 neutropenia and G2 anemia were observed in 10% and 5, respectively.

Conclusion:

Navelbine & carboplatin followed by oral navelbine is an effective, safe and tolerable regimen in patients with metastatic/recurrent TNBC previously treated with adjuvant anthracycline and taxane with ORR 75%.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-16

Title: Utilization and outcomes of eribulin in triple negative metastatic breast cancer: Real-world findings

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Body: Background

Triple-negative breast cancer (TNBC) accounts for 10-20% of all breast cancers (BCs) and a significant proportion of all BC deaths. Eribulin is approved for the treatment of metastatic BC (MBC) after treatment with two prior regimens. A pooled analysis of two phase III studies of eribulin in women with TNBC patients found a 26% reduction in the risk of death vs. controls. Treatment patterns of eribulin and clinical outcomes associated with early vs. late use among TNBC patients treated in community oncology practices have not been evaluated.

Methods

Physicians from the Cardinal Health Oncology Research Network completed an electronic case report form (CRF) on up to 7 TNBC patients treated with eribulin between 01/01/11 and 12/31/13. Adult female patients with pathologically confirmed metastatic disease and not participating in any interventional clinical trial were included. Providers indicated the usage of chemotherapy, either alone or in combination, by line of therapy (LOT) up to the LOT of eribulin initiation. Reported data points include: clinical parameters (eg, site of metastases, ECOG performance status, and comorbidities), treatment events (eg, LOT start/end date and rationale for discontinuation), and outcomes (eg, clinical response and date of death). Dosing, adverse events, use of supportive care medications, and hospitalization were also captured during eribulin treatment. Use of eribulin in LOT 1/LOT 2 was considered early; LOT 3+ was considered late. All comparisons are univariate.

Results

An interim analysis was performed on 123 TNBC patients (planned sample size of 250) collected from 26 providers. Patient mean age at eribulin treatment initiation was 55.0 years. Mean follow-up duration was 27 mo (SD = 11.9) from initiation of first line metastatic treatment until date of last visit, death, or loss to follow-up. Overall, 74.0% were deceased, 85.4% had received at least 3 LOTs in the metastatic setting, and 45.4% were stage IV at diagnosis. Most women were prescribed eribulin in a later LOT (61.8%), 3 (2.4%) patients received eribulin in LOT1 and 44 in LOT2 (36.7%). Among patients with known treatment start and end dates (87.0%), mean duration of treatment (DOT) was 6.2 mo (SD = 3.3), median 5.8 mo among early recipients and 5.5 mo (SD = 5.7), median 4.1 mo, among later recipients ($p = 0.39$). Early users were more likely ($p = 0.05$) to have a complete/partial response (71.1% vs. 47.7%) and less likely to have progressive disease (7.1% vs. 12.3%). In comparing eribulin users to all other therapies, eribulin users had a significantly longer DOT in LOT2 (5.9 vs. 4.7 mo, $p = 0.01$) and LOT3 (5.8 vs. 3.6 mo, $p = 0.03$). In LOT3, eribulin users were significantly more likely to have complete/partial response (54.2% vs. 18.8%) and less likely to have to have progressive disease (4.2% vs. 37.5%) compared to all other observed LOT3 therapies.

Conclusions

This interim analysis indicates longer DOT for patients treated with eribulin for TNBC in LOT2 and LOT3 and a more favorable response rate compared to all other agents used in each LOT, respectively, among patients treated in community oncology practices. Full results will be available at the conference.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-17

Title: Platinum salts in advanced breast cancer: A systematic review and meta-analysis of randomized clinical trials

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Body: Background The interest in platinum salts for breast cancer (BC) treatment has been recently renewed as inhibition of DNA damage response may enhance the effects of DNA-damaging agents in tumors with high genomic instability. The present systematic review and meta-analysis of randomized trials was performed to assess the efficacy and safety of platinum salts in patients with advanced BC. **Methods** We searched Pubmed, EMBASE, SCOPUS, Web of Science, the Cochrane Library and CINAHL for phase II/III clinical trials that assessed efficacy of platinum-based therapy in patients with advanced BC. Pooled estimates of overall response rate (RR), median progression-free survival (PFS) and overall survival (OS) and major toxicities were computed by using random or fixed effects models. **Results** A total of 4,625 patients from 23 phase II/III trials (11 with cisplatin, 11 with carboplatin and 1 with either agents) were analyzed. Estimates for RR, PFS and OS were obtained from 23, 13 and 15 studies, respectively. Though at the price of increased hematological, gastrointestinal toxicity and fatigue, compared with non platinum-salts schemas, cisplatin and carboplatin prolonged OS (HR 0.91; 95% CI 0.83 to 1.00, p= 0.04), PFS (HR 0.84; 95% CI 0.73 to 0.97, p= 0.01), and RR (HR 1.27; 95% CI 1.03 to 1.57, p=0.03). **Conclusions** Despite some limitations of the studies examined, including the lack of information on hormonal receptor and HER2 status, the use of platinum salts significantly improved RR, PFS and OS of patients with advanced BC with no unexpected toxicity.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-15-18

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-01

Title: A randomised phase III trial comparing two dose-dense, dose-intensified approaches (ETC and PM(Cb)) for neoadjuvant treatment of patients with high-risk early breast cancer (GeparOcto)

Schneeweiss A, Möbus V, Tesch H, Hanusch C, Denkert C, Lübke K, Huober J, Klare P, Kümmel S, Untch M, Kast K, Jackisch C, Ingold-Heppner B, Thomalla J, Blohmer J-U, Rezai M, Nekljudova V, von Minckwitz G and Loibl S. Universitätsklinikum Heidelberg; Klinikum Frankfurt-Höchst; Hämatologisch-Onkologische Gemeinschaftspraxis, Frankfurt; Klinikum zum Roten Kreuz, München; Charite Berlin; Diakovere Henriettenstift Hannover; Universitätsklinikum Ulm; Praxisklinik Krebsheilkunde für Frauen/Brustzentrum Berlin; Kliniken Essen- Mitte, Essen; Helios Kliniken Berlin-Buch; Universitätsklinikum Dresden; Sana Klinikum Offenbach; Praxisklinik für Hämatologie und Onkologie Koblenz; Luisenkrankenhaus Düsseldorf and German Breast Group, Neu-Isenburg.

Body: Background: The sequential use of dose-dense, dose-intensified epirubicin, paclitaxel, and cyclophosphamide (EPC) (Möbus et al. J Clin Oncol 2010) as well as weekly treatment with paclitaxel/liposomal doxorubicin (plus carboplatin in triple negative disease, TNBC) (PM(Cb)) (von Minckwitz et al. Lancet Oncol 2014), both with dual HER2 blockade in HER2+ breast cancer (BC), are considered highly efficient regimens for high-risk early stage BC patients and will be compared in the GeparOcto study.

Methods: GeparOcto (NCT02125344) patients received either 18 weeks EPC (E 150mg/m² q2w for 3 cycles, P 225mg/m² q2w for 3 cycles, C 2000mg/m² q2w for 3 cycles) or PM(Cb) (P 80mg/m² q1w concomitantly with M 20mg/m² q1w and, in case of TNBC, Cb AUC 1.5 q1w). Patients with HER2+ BC received trastuzumab 6 (8) mg/kg q3w and pertuzumab 420 (840) mg q3w concomitantly with all P, C, and PM(Cb) cycles. Due to a high risk of chemotherapy induced anaemia in both regimens, patients developing iron deficiency anaemia during the study were also randomized to parenteral ferric carboxymaltose or physician's choice for treatment.

Patients with untreated, histologically confirmed, cT1c - cT4a-d BC and central receptor assessment were included. Patients with HER2+ or TNBC were eligible irrespective of nodal status, luminal B-like tumours only if pN+. Primary objective is to compare pCR rates (ypT0/is ypN0). Sample size calculations assumed a pCR rate of 50% for EPC and 60% for PM(Cb), requiring 950 patients to show superiority of PM(Cb). Secondary objectives are to compare pCR rates between treatment arms within the stratified subpopulations (BC subtype [HER2+ vs HER2- HR+ vs HER2- HR-], Ki-67 [≤ 20 vs $> 20\%$], lymphocyte-predominant BC [$\geq 60\%$ tumor infiltrating lymphocytes (TILs), no vs yes]), amongst others.

Results: 961 patients were recruited between 12/2014 and 05/2016, 946 started treatment. Median age was 48/48 years (EPC/PM(Cb)), 2/2% had bilateral, 13/16% multifocal, 7/7% multicentric, 4/4% T3, 4/4% T4, 46/47% N+, 81/83% ductal invasive, 65/68% G3 tumors; 41/40% were HER2+, 43/43% TNBC. 286 patients reported SAEs (147 EPC/139 PM(CB)) and 1 died (1 PMCb: multiorgan failure due to sepsis). Final analysis on primary endpoint will be presented.

Conclusions: GeparOcto investigates the efficacy of dose-dense dose-intensified EPC compared to weekly PM(Cb), both with dual HER2-blockade in HER2+ BC, in high-risk early stage breast cancer patients.

The trial is financially supported by Roche, Amgen, Teva and Vifor.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-02

Title: Pathological complete response is associated with excellent outcomes in *BRCA* mutation associated triple negative breast cancer

Prochaska LH H, Godwin AK K, Kimler BF F, Lehn C, Klemp JR R, O'Dea A, Elia M, Hoffmann MS S, Crane G, McKittrick R, Sheehan M, Graff SL L, Madhusudhana S, Khan QJ J, Jensen RA A and Sharma P. University of Kansas Medical Center, Westwood, KS; Sarah Cannon Cancer Center, Overland Park, KS and Truman Medical Center, Kansas City, MO.

Body: Introduction: Pathological complete response (pCR) in unselected triple negative breast cancer (TNBC) is associated with excellent long-term survival. However, controversy remains as to whether pCR in *BRCA* mutation associated (BRCA[+]) TNBC is predictive of improved long-term outcome. A recent study suggests that pCR was not a surrogate for outcomes in *BRCA1* associated TNBC. All of the patients in this study harbored an Ashkenazi Jewish founder *BRCA1* mutation and the majority of mutation carriers underwent lumpectomy. Impact of pCR as it relates to *BRCA* status in a larger, heterogeneous TNBC cohort treated in a contemporary time frame is not known.

Aim: Evaluate and compare the prognostic impact of pCR as it relates to the *BRCA* mutation status in patients enrolled in a prospective multisite TNBC registry.

Methods: 453 patients with stage I-III TNBC were enrolled within a multisite registry between 2011- 2015, out of which 173 received neoadjuvant chemotherapy (NAC) and also underwent germline *BRCA* testing. pCR in the breast and axilla was evaluated and patients were followed for reoccurrence and survival. Recurrence free survival (RFS) was estimated according to the Kaplan-Meier method and compared among groups with log-rank statistic.

Results: For the 173 eligible patients the median age was 49 years; African-American:14%; median tumor size:3 cm; 42%:Lymph node positive; and 18% (32/173) demonstrated *BRCA* mutation (*BRCA1*=28, *BRCA2*=4). All patients received anthracycline and/or taxane based NAC. pCR rates for BRCA[+] and wild type (BRCA[-]) patients was 72% and 46% respectively (p=0.01). 97% of BRCA[+] and 42% of BRCA[-] patients underwent bilateral mastectomy (p=0.001). The three year RFS was 92% and 81% in BRCA[+] and BRCA[-] patients, respectively (p=0.18). Attainment of pCR was associated with excellent 3 year RFS of 95% and 97% in BRCA[+] and BRCA[-] patients, respectively (p=0.85). Among BRCA[-] patients lack of pCR was associated with significantly worse 3 year RFS (70% RFS in patients without pCR, compared to 97% in patients with pCR; p=0.001). Among BRCA[+] patients lack of pCR was associated with numerically lower but not statistically significant worse 3 year RFS (83% RFS in patients without pCR, compared to 95% in patients with pCR; p=0.41). On multivariable Cox regression analysis, only stage III disease was associated with higher risk of relapse (p<0.001).

Conclusions: Our observation of higher pCR in *BRCA*-carriers compared to wild-type TNBC patients is consistent with previously published literature. In this contemporary cohort of TNBC patients for whom the majority of BRCA[+] patients underwent bilateral mastectomy, attainment of pCR carried an excellent prognosis in both BRCA[+] and BRCA[-] patients. On the other hand, BRCA[+] patients who do not attain pCR may have better outcomes compared to BRCA[-] patients without pCR. Further research to explore the underlying biological mechanisms involved in tumor response and relapse in BRCA[+] and BRCA[-] TNBC patients is needed. Furthermore, given these observations, germline *BRCA* mutation status should be used as a stratification variable in studies evaluating pCR and long term outcomes with investigational therapies in TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-03

Title: Peripheral sensory neuropathy occurrence and resolution: Results from the neoadjuvant randomized GeparSepto study (GBG 69)

Furlanetto J, von Minckwitz G, Jackisch C, Schneeweiss A, Aktas B, Denkert C, Wiebringhaus H, Kuemmel S, Warm M, Paepke S, Just M, Hanusch C, Hackmann J, Blohmer J-U, Clemens M, Costa SD Dan, Gerber B, Nekljudova V, Untch M and Loibl S. German Breast Group, Neu-Isenburg; Sana Klinikum Offenbach; Universitätsklinikum Heidelberg; Universitätsklinikum Essen; Charite Berlin; St. Barbara Kliniken Heessen Hamm; Kliniken Essen-Mitte, Essen; Kliniken der Stadt Köln GmbH, Köln; Frauenklinik und Poliklinik der Technischen Universität München; Onkologische Schwerpunktpraxis Bielefeld; Klinikum zum Roten Kreuz, München; Marien Hospital Witten; Mutterhaus der Borromäerinnen Trier; Universitätsklinikum Magdeburg; Universitätsklinikum Rostock and Helios Kliniken, Berlin-Buch.

Body: Background: The GeparSepto (NCT01583426) study showed that nab-paclitaxel (nP) increases the pathological complete response (ypT0 ypN0) rate when it replaces paclitaxel (P) as part of a sequential taxane followed by epirubicin/cyclophosphamide (EC) neoadjuvant chemotherapy for pts with early breast cancer (BC) (Untch Lancet Oncol 2016). After a safety analysis showed a higher rate of dose reductions, treatment discontinuations as well as peripheral sensory neuropathy (PSN) with nP 150 mg/m² w (nP150) compared to P 80mg/m² w, dose of nP was reduced to 125 mg/m² w (nP125). The risk-benefit ratio of nP125 was improved over nP150 (von Minckwitz SABCS 2015). We reported follow-up (FU) data on PSN occurrence and resolution.

Methods: Pts with untreated BC received P 80mg/m² w or nP 150/125mg/m² w followed by four cycles of E 90 mg/m² plus C 600 mg/m² q3w, with trastuzumab 6 mg/kg (loading (LD) dose 8 mg/kg) and pertuzumab 420 mg (LD 840 mg) q3w if HER2+. After the end of the study the protocol was amended in order to collect long-term data on PSN outcome as well as on treatment modalities. PSN will be reported according treatment and dose received on day 1.

Results: Overall 601 pts received P80; 220 pts nP150 and 385 pts nP125 on day 1. PSN grade 2-4 was observed in 18.8% (n=113/601) of pts treated with P80 and in 41.8% (n=92/220) vs 39.2% (n=151/385) with nP150 and nP125 respectively (p=0.547). Grade 3-4 PSN was reported for 2.7% (n=16/601) of pts in the P80 group and 14.5% (n=32/220) vs 8.1% (n=31/385) in the nP150 vs nP125 group respectively (p=0.018). In 31.8% (36/113), 35.9% (33/92) and 27.2% (41/151), PSN was not resolved at the end of the treatment (EOT); PSN grade 3-4 was not resolved in 37.5% (6/16), 56.3% (18/32) and 58.1% (18/31). After a median FU of 110 weeks after EOT, data on PSN status for pts with unresolved PSN grade 2-4 were available from 30, 22 and 32 pts; 26 pts did not provide update information (n=7 died, n=5 data not yet available, n=14 status unknown). For 63.3% (n=19), 40.9% (n=9) and 56.2% (n=18) of pts, PSN grade 2-4 was resolved to grade 1. Time to resolve (TTR) of PSN grade 2-4 was significantly different between nP150 and nP125 (p<0.001); no significant difference was seen between P and nP (p=0.405) [Tab.1]. After a median FU of 103 weeks after EOT, data on PSN status of pts with unresolved PSN grade 3-4 were available for 6, 14 and 14 pts. For 66.6% (n=4), 42.8% (n=6) and 50.0% (n=7) of pts PSN grade 3-4 was resolved to grade 1. TTR of PSN grade 3-4 was not significantly different neither for nP150 vs nP125 (p=0.103) nor for P vs nP (p=0.120)) [Tab.1].

Conclusions: nP125 is associated with a lower occurrence of PSN compared to nP150 but higher PSN than P80. If PSN occurred nP125 is associated with a more rapid resolution compared to nP150. Nearly 10.7% had no resolution of PSN so far. Further FU and markers for selecting pts at risk are needed.

The trial is supported by Celgene.

Table 1. Median time to resolution (mTTR) of PSN to grade 1

comparison groups	mTTR n (weeks); [95% CI]		
	P	nP150	nP125
P vs nP			
grade 2-4	7 [6-9]	8 [6-10]	
grade 3-4	9 [4-15]	17 [5-123]	
nP150 vs nP125			
grade 2-4		13 [9-15]	6 [4-9]

grade 3-4		56 [11-170]	17 [10-nr]
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abbreviations: nP, nab-paclitaxel; P, paclitaxel; nr, not reached

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-04

Title: A randomized phase II neoadjuvant study comparing docetaxel and cyclophosphamide (TC) with 5-fluorouracil, epirubicin, and cyclophosphamide followed by docetaxel (FEC-D) for hormone receptor-negative breast cancer: The Kanagawa breast oncology group (KBOG) 1101 study

Narui K, Ishikawa T, Shimizu D, Tanabe M, Sasaki T, Oba MS Saito, Morita S, Nawata S, Kida K, Mogaki M, Doi T, Tsugawa K, Ogata H, Ota T, Kosaka Y, Sengoku N, Kuranami M, Saito Y, Suzuki Y, Suto A, Arioka H, Chishima T, Ichikawa Y, Endo I and Tokuda Y. Yokohama City University Medical Center, Yokohama, Japan; Tokyo Medical University, Tokyo, Japan; Yokosuka Kyosai Hospital, Yokosuka, Japan; Shonan Kinen Hospital, Kamakura, Japan; St. Marianna University School of Medicine, Kawasaki, Japan; Kitasato University, Sagami-hara, Japan; Tokai University, Isehara, Japan; Yokohama Rosai Hospital, Yokohama, Japan and Yokohama City University, Yokohama, Japan.

Body: Purpose: This study aimed to evaluate response to neoadjuvant chemotherapy (NAC) for patients with hormone receptor-negative (HR-negative) breast cancer (BC) to identify subtypes that require anthracycline treatment.

Methods: In total, 103 patients with operable HR-negative BC were registered. They were randomly assigned to administration of 6 cycles of docetaxel (75mg/m²) and cyclophosphamide (600 mg/m²) (TC6) or 3 cycles of 5-fluorouracil (500 mg/m²), epirubicin (100mg/m²), and cyclophosphamide (500mg/m²) followed by 3 cycles of docetaxel (100mg/m²) (FEC-D). Cytokeratin (CK) 5/6 and EGFR expression were used to identify basal and non-basal triple-negative (TN) BC. The primary endpoint was pathological complete response (pCR); secondary endpoints were safety, breast-conserving surgery, disease-free survival, and overall survival. Predictive factors of pCR for each regimen were also evaluated.

Results:

The pCR rate was 36% for FEC-D and 25.5% for TC6, which did not differ significantly ($P=0.265$). When TN BC was subdivided into basal and non-basal subtypes, the pCR rate in the basal subtype was significantly lower for TC6 (13.6%) than for FEC-D (42.9%) ($P=0.033$), but did not significantly differ in the non-basal (TC6, 36.4%; FEC-D, 25.0%) and HER2-positive (TC6, 41.7%; FEC-D, 35.7%) cases.

The relative dose intensities of epirubicin and docetaxel in FEC-D and docetaxel in TC6 were $96.3\pm 13.0\%$, $93.5\pm 14.6\%$, and $93.9\pm 16.3\%$ (mean \pm SD), respectively. Occurrence of grade ≥ 2 adverse events was significant in FEC-D-treated patients. Poor appetite ($P<0.001$), nausea ($P<0.001$), vomiting ($P<0.001$), dysgeusia ($P=0.03$), and fatigue ($P=0.05$) were significantly more common for FEC-D than TC6. Patients treated with FEC-D experienced significantly more febrile neutropenia and anemia ($P=0.016$ and 0.017 , respectively).

The rates of breast-conserving surgery were 68.0 and 72.3% for FEC-D and TC6, respectively ($P=0.641$).

Patients achieved pCR had better DFS (log rank test, $P = 0.287$) and OS (log rank test, $P = 0.069$), though not significant. Patients treated with FEC-D had better DFS (log rank test, $P = 0.107$) and OS (log rank test, $P = 0.159$), though not significant. Among patients with TN BC, those treated with FEC-D had significantly better DFS (log rank test, $P = 0.016$) and OS (log rank test, $P = 0.034$) than treated with TC6.

Low ALDH1 expression and high topo II α protein expression were strongly correlated with pCR in FEC-D, with odds ratios (ORs) of 4.33 [95% CI, 1.02–18.38] and 4.08 [0.97–17.2], respectively. ALDH1 was also associated with pCR in TC, OR=3.50 [0.84–14.6]. Other factors, including age, tumor size, nodal status, tumor grade, Ki67, p53, and *TOP 2A* status were not associated with pCR in either regimen.

Conclusions: We found that TC6 was less effective than FEC-D for treating HR-negative BC because it was insufficient for TNBC, particularly for basal BC. This suggests that anthracycline is more important than taxane for basal BC. Additionally, ALDH1 could be a marker for resistance to conventional chemotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-05

Title: MINT trial yields MammaPrint High1/High2 risk classes associated with significant differences in pCR and receptor subtype

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Body: Background: Previous clinical trials have validated that the 70-gene signature MammaPrint™ provides prognostic and predictive information for early stage breast cancer patients and can identify low risk patients who may safely avoid adjuvant chemotherapy. Additionally, the neo-adjuvant I-SPY 1&2 TRIALS demonstrated that further stratification of patients into MammaPrint High 1 (MP1) and MammaPrint High 2 (MP2) risk groups may help predict chemo-sensitivity. There were significant differences in pathological complete response (pCR) rates for early stage, locally advanced breast cancer patients who were not HR+HER2- MammaPrint Low Risk. Specifically, the PARP inhibitor veliparib in combination with carboplatin recently graduated the I-SPY 2 phase 2 screening trial, having met the 85% predictive probability criterion with a triple-negative breast cancer signature, which was the subset recommended for this regimen's subsequent development. Given these data, we wanted to determine whether the Multi Institutional Neo Adjuvant Therapy MammaPrint Project (MINT) patient population confirmed the MP1/MP2 risk stratification, clarify if there is an associated receptor subtype for MP1/MP2 risk classes, and conclude if the stratification correlates to a significant difference in pCR. **Methods:** Array data from pre-treatment samples were obtained from 180 patients classified as MammaPrint High Risk, subtyped by IHC and treated with neo-adjuvant chemotherapy according to protocol. Response was measured by centrally assessed residual cancer burden pursuant to guidelines. Patients were then further stratified based on the MammaPrint Index per their classification threshold between MP1/MP2. Fisher's exact test was used to assess significance of association with pCR overall and within hormone receptor (HR) and HER2 subtypes. **Results:** MP1 vs MP2 risk classes yielded subsets with significant (p=0.007) differences in pCR. 44% (40/92) of MP2 patients achieved a pCR, compared to 24% (21/88) of MP1 patients. Next, we investigated whether the MP1 and MP2 risk classes were associated with receptor subtype. MP1 demonstrated a significant association and MP2 near significance. 32% (21/66) of triple-negative patients were classified as MP2 vs only 3% (2/66) MP1. Similarly, in the overall population, 28% (51/180) HR+HER2- are classified as MP1 vs 4% (8/180) MP2. Results in the pCR population were reflective of these subtype trends. 63% (58/92) of MP2 patients were classified triple-negative, of which nearly one quarter (21/92) had a measured pCR, whereas 58% (51/88) of MP1 patients were HR+HER2- with 3% (3/88) achieving pCR (Table1). **Conclusion:** This analysis in the MINT patient population supports previously published data and suggests that the MammaPrint High 1/2 risk classification may help predict chemo-sensitivity. Given the statistical significance of these data, we are currently investigating the biological mechanisms distinguishing the MP1/MP2 subgroups that may account for its use as a specific biomarker of response to chemotherapy treatment in future trials.

Table 1.

MP1				MP2			
HER2+		HER2-		HER2+		HER2-	
HR+	HR-	HR+	HR-	HR+	HR-	HR+	HR-
7	9	3	2	4	13	2	21
8	5	48	6	4	5	6	37
15	14	51	8	8	18	8	58

Row#1= pCR, #2= RD, #3= Total

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-06

Title: Neoadjuvant weekly carboplatin and paclitaxel followed by dose dense epirubicin and cyclophosphamide in triple negative breast cancer patients: A single arm phase II study from the Belgian Society of Medical Oncology

Fontaine C, Cappoen N, Renard V, Vuylsteke P, Van Den Bulck H, Glorieux P, t'Kint de Roodenbeke D, Dopchie C, Decoster L, Vanacker L, De Grève J, Awada A and Wildiers H. UZ Brussel, Jette, Brussels, Belgium; University Hospitals Leuven, Leuven, Belgium; Belgian Society of Medical Oncology (BSMO), Jette, Brussels, Belgium and Jules Bordet Institute, Brussels, Belgium.

Body: Introduction: Triple negative breast cancer (TNBC) remains a challenging disease with dismal prognosis. Platinum analogs have not yet shown to improve long term outcome in this setting, but are associated with increased pathological complete response rate (pCR) at the cost of higher toxicity.

Aim: To further increase or maintain the high pCR rate with platinum containing schedules while decreasing toxicity by administering low dose weekly carboplatin instead of high-dose 3 weekly carboplatin as in CALGB 40603.(1)

Patients and methods: We evaluated the tolerability and the impact of the addition of *weekly* carboplatin (CP) to paclitaxel (P) and dose dense epirubicin-cyclofosfamide (EC) on pCR in an open-label multicenter phase II study in stage II/III TNBC patients (pts). Sixty three pts received dose dense paclitaxel (P:80mg/m²/wk) concurrent with carboplatin (CP: AUC=2) for 12 wks, followed by two-weekly epirubicin (E:90mg/m²) and cyclophosphamide (C:600mg/m²) for 4 cycles. The primary endpoint is pCR in the breast and axilla. Additionally treatment delivery and adverse events are recorded. A correlative assessment of germline mutations in homologous recombination (HR) genes is planned. Pts are monitored for response by magnetic resonance and mammography and also for relapse free survival and time to treatment failure. The study size sample has been calculated according to the optimal Simon's two-stage design method. The target sample size was 63 patients with 80% power to detect a pCR rate of ≥47% ($\alpha= 0.05$).

Results: Accrual to the study is completed with 63 eligible pts with operable, noninflammatory stage II and III TNBC included. Most patients were between 40 and 60 yrs old and were clinical stage T2 tumors. Half of the pts were clinically node + and 70% were G3. Sixty six percent had breast conserving surgery. Sixteen out of 26 (61.5%) of the currently evaluable pts achieved a pCR rate in the breast and axilla. The other ongoing patients have not yet reached this endpoint. Four out of 21 evaluable pts that completed the chemotherapy missed two or more doses of CP due to neutropenia (NP) G3/4(2), general deterioration G3(1) and polyneuropathy (PNP) G3(1) and seven pts needed one dose reduction of P and/or CP due to NP G3-4 (3-2) and PNP G2(1) and one abdominal infection.

Conclusion: These preliminary data suggest that the addition of *weekly* carboplatin to neoadjuvant paclitaxel and EC is feasible and has a promising pCR rate in the breast and axilla as high as 61.5% in early TNBC pts. More mature toxicity and outcome data and correlation with genome analysis will be presented.

(1) Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once per week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance) Sikov WM et al. J Clin Oncol 33:13-21; 2014.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-07

Title: Non-anthracycline-containing docetaxel plus cyclophosphomide was inferior to docetaxel, anthracycline and cyclophosphomide in neoadjuvant treatment of triple negative or HER2 positive breast cancer: Long term follow-up result from NATT study

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Body: Objective: Previous study demonstrated that non-anthracycline-containing docetaxel plus cyclophosphomide (TC) regimen was associated with lower response rate and worse disease outcome compared with docetaxel, anthracycline and cyclophosphomide (TAC) regimen in neoadjuvant treatment of triple negative or HER2 positive breast cancer in terms of short term survival outcome. In this study, we evaluate the long-term survival outcome between TC and TAC.

Methods: Clinical stage IIB or III TNBC or HER2+ breast cancer patients were treated with six cycles of TC or TAC. The primary end point was pathological complete remission (pCR). Secondary end points included clinical response rate, event free survival (EFS), disease free survival (DFS), and overall survival (OS).

Results: 102 patients were randomized and 96 patients were available for this data analysis (TC: n=45; TAC: n=51). TAC regimen had a trend of higher pCR rate than TC regimen: 17.6% vs. 6.8%, $P = 0.113$. With a median follow-up of 53 (8-76) months, patients achieved pCR after neoadjuvant chemotherapy had a superior EFS, DFS and OS compared with patients without pCR ($P < 0.05$). TAC treatment resulted in a significantly superior outcome than TC treatment: the estimated 5-year EFS was 68.1% vs 29.8%, $P = 0.001$; the estimated 5-year DFS was 69.7% vs 34.0%, $P = 0.001$; and the estimated 5-year OS was 88.4% vs 51.6%, $P < 0.001$. Multi-variable survival analysis demonstrated that treatment regimen was the independent prognostic factor for these patients. Patients treated with TAC had a superior EFS, HR = 0.40 (95% CI 0.21-0.75), $P = 0.004$; a better DFS, HR = 0.40 (95% CI 0.21-0.78), $P = 0.007$; and a better OS, HR = 0.19 (95% CI 0.07-0.57), $P = 0.002$.

Conclusions: Long term follow-up data demonstrated that adding anthracycline to TC could significantly improve disease outcome, indicating an anthracycline should be considered as a necessary and effective drug in this trial setting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-08

Title: Risk factors of locoregional relapse in locally advanced breast cancer treated with neoadjuvant chemotherapy following mastectomy and radiotherapy

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Body: Purpose: We seek to investigate the prognostic factors that could possibly affect the local-regional recurrence (LRR) of breast cancer patients who do not achieve pathological complete response (pCR) after neoadjuvant chemotherapy (NCT), and to build a prognostic nomogram to predict patients' outcome.

Methods: The retrospective analysis included 510 patients who had received NCT followed by surgery and radiotherapy. The NCT regimens included anthracycline-containing, vinorelbine-containing, and taxane-containing regimens for a median of 3 cycles. The clinical responses to neoadjuvant chemotherapy were evaluated based on MRI and ultrasound examinations and in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. The Miller-Payne (MP) grading system was employed to evaluate the decrease in cancer cellularity. Multivariate analyses were used to identify independent predictors of LRR.

Results: The median follow-up for the entire study population was 61 months (range 5-128 months). There were 40 isolated local recurrences, 17 regional-only recurrences and 5 patients with local and regional recurrences. Sixteen patients (25.8%) occurred synchronously with distant metastasis, and no LRR was detected after distant failure. Fifteen patients had recurrences in the supraclavicular field. The 5-year cumulative LRR-free survival rate was 88.0% (95% CI: 85.2%-90.1%). The 5-year overall survival and disease-free survival rates were 79.0% (75.5%-82.6%) and 63.1% (58.9%-67.3%), respectively. Multivariate analysis revealed that positivity for ≥ 4 lymph nodes and Ki-67 index $\geq 14\%$ were independent factors for LRR-free recurrence. The prognostic model was established based on the sum of both independent prognostic factors, with positivity for ≥ 4 lymph nodes and Ki-67 index $\geq 14\%$ individually contributing 1 point to the risk score. The patients in the validation set were assigned to a low-risk group (0 point), median-risk group (1 point) and high-risk group (2 points). The 5-year LRR-free survival rates in the low, intermediate, and high-risk groups were 95.5%, 89.1%, and 67.1%, respectively ($p < 0.001$). We also show the annual LRR rate among these 3 groups using our single-institution data, as well as to review relevant literature-based data to compare with our observations. Four publications that reported ARR (annual recurrence rate) after surgery for primary breast cancer were identified as eligible. In the relevant literature, the annual risk of local-regional recurrence peaked between two and three years after the initial diagnosis. In contrast, the ARR curve of the high risk subgroup for neoadjuvant patients exhibited one peak near 1 year (17% per annum). The median and low-risk subgroups did not have an obvious recurrence peak.

Conclusion: This prognostic model has considerable clinical value in predicting local-regional recurrence, which could help clinicians to design appropriate local-regional treatment specifically and to perform surveillance individually.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-09

Title: Overall survival of patients with non-metastatic triple negative breast cancer who received neoadjuvant vs adjuvant chemotherapy: Cohort analysis of National cancer data base (NCDB) 2010 - 2011

Cheng YC, Smith E and Yen T. Medical College of Wisconsin, Milwaukee, WI.

Body: Background: One of the benefits of neoadjuvant approach to the treatment of breast cancer is early microscopic disease control, which should translate to improved survival. However, clinical trials have not yet shown a survival benefit for neoadjuvant approach in even high risk patients, such as triple negative cases. Few studies have been performed outside of clinical trials.

Purpose: The objective of our study was to compare the overall survival of Stage I-III triple negative breast cancer patients who received neoadjuvant vs adjuvant chemotherapy within the NCDB, a prospectively collected, large, nationwide, hospital-based cancer outcomes database which contains information for more than 1,500 Commission on Cancer-accredited cancer programs in the U.S.

Patient and Method: We identified a cohort of women, aged ≥ 18 year-old at diagnosis, with clinical stage I-III triple negative breast cancer diagnosed in 2010-2011, who received either neoadjuvant chemotherapy only or adjuvant chemotherapy only. Patients with incomplete or missing vital status, receptors status and treatment information were excluded. Demographic (age at diagnosis, race, ethnicity, comorbidities, insurance, median income, urbanicity), tumor (clinical stage, histology, grade) and treatment (breast surgery, surgical margin, radiation) factors were examined. Stabilized inverse proportion weights were developed and assigned to balance the neoadjuvant and adjuvant groups on all demographic, tumor and treatment covariates. Unadjusted and adjusted overall survival was calculated using the Kaplan-Meier method and compared using the log-rank test.

Results: Among the 15,483 women with triple negative breast cancer, 4,335 (28%) received neoadjuvant chemotherapy and 11,148 (72%) received adjuvant chemotherapy. Most of the demographic, tumor and treatment factors were similarly distributed among neoadjuvant and adjuvant groups except age at diagnosis and clinical stage. Compared to patients received adjuvant chemotherapy, patients received neoadjuvant chemotherapy were more likely to be younger (45% vs. 31% < 50 year-old, $p < 0.0001$) and have a higher clinical stage (35% vs. 6% stage III, $p < 0.0001$). The unadjusted 4-year overall survival of patients received neoadjuvant vs adjuvant approach was 75.8% (95% CI 74.4%-77.4%) and 87.5% (95% CI 87.1%-87.9%), respectively. After adjusting for demographic, tumor and treatment factors, the 4-year overall survival of patients received neoadjuvant vs adjuvant approach was 81.9% (95% CI 79.5%-84.3%) and 85.3% (95% CI 85.2%-85.4%), respectively.

Conclusion: In this NCDB study, the overall survival of triple negative breast cancer patients received neoadjuvant chemotherapy was inferior to those received adjuvant chemotherapy, even after adjusting for demographic, tumor and treatment factors. However, information regarding the chemotherapy regimen used and whether a full course of chemotherapy was delivered (2 factors that affect disease response and outcome) was not available. Patient and tumor factors at the time of disease presentation that are important in determining which triple negative patients will benefit from neoadjuvant approach remain to be defined.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-10

Title: Zoledronic acid combined with neoadjuvant chemotherapy for HER2-negative early breast cancer (JONIE 1 trial): Survival outcomes of a randomized multicenter phase 2 trial

Ishikawa T, Akazawa K, Hasegawa Y, Tanino H, Horiguchi J, Miura D, Hayashi M, Takao S, Kim SJ, Yamagami K, Miyashita M, Konishi M, Shigeoka Y, Suzuki M, Taguchi T, Kubota T and Kohno N. Tokyo Medical Univeristy, Tokyo, Japan; Niigata University Medical and Dental Hospital, Niigata, Japan; Hirosaki Municipal Hospital, Hirosaki, Japan; Kitasato University Hospital, Sagami-hara; Gunma University Hospital, Maebashi, Japan; Toranomon Hospital, Tokyo, Japan; Tokyo Medical University Hachioji Medical Center, Tokyo, Japan; Hyogo Cancer Center, Kobe, Japan; Osaka University, Osaka, Japan; Shinko Hospital, Kobe, Japan; Konan Hospital, Kobe, Japan; Hyogo Prefectural Nishinomiya Hospital, Kobe, Japan; Yodogawa Christian Hospital, Osaka, Japan; National Hospital Organization, Chiba Medical Center, Chiba, Japan; Kyoto Prefectural University of Medicine, Kyoto, Japan; Kamiida Daiichi General Hospital, Nagoya, Japan and Kobe Kaisei Hospital, Kobe, Japan.

Body: BACKGROUND and AIM:

Findings from a randomized phase 2 JONIE1 trial in women with HER2-negative early breast cancer have shown that the addition of zoledronic acid (ZOL) to neoadjuvant chemotherapy (CT) has potential anticancer benefits in postmenopausal and triple-negative breast cancer patients. We report the data for the prespecified secondary endpoint of disease-free survival (DFS).

METHODS:

We enrolled women with HER2-negative early breast cancer and randomly assigned them to receive CT or CT+ZOL (CTZ). All patients received 4 cycles of FEC100 (fluorouracil 500 mg/m², epirubicin 100 mg/m², and cyclophosphamide 500 mg/m²), followed by 12 cycles of paclitaxel at 80 mg/m² weekly. ZOL (4 mg) was administered 3-4 times weekly for 7 weeks to the CTZ group patients. Definitive surgery was performed 3-4 weeks after the last paclitaxel dose. The primary endpoint was pathological complete response (pCR). The secondary endpoints were the clinical response rates, rate of breast-conserving surgery, safety, and DFS (defined as the time from randomization to disease occurrence or death). The trial is registered as UMIN000003261 (www.umin.ac.jp/english/) with ongoing follow-up.

FINDINGS:

Of the 188 patients enrolled, 95 were assigned to the CT group and 93 to the CTZ group. The mean (95% CI) DFS time of the CT group was 5.15 years (4.83-5.47) and that of the CTZ group was 5.38 years (5.11-5.66). The 3-year DFS rate was 84.6% (95% CI 77.2-92.0) in the CT group and 90.7% (84.6-96.8) in the CTZ group with no significant difference ($p = 0.120$). The particular benefit from ZOL for the neoadjuvant CT seen as improvement of the pCR rate was indicated in the 3-year DFS period for triple-negative cancer cases (CT vs CTZ: 70.6% vs 94.1%), but not for postmenopausal cases.

CONCLUSIONS:

ZOL slightly improved DFS when combined with CT. Although a significant difference was not found in this study, plans are underway for conducting a combined analysis of 3 neoadjuvant CT trials together with ZOL. The improvement of the pCR rate may be associated with DFS in triple-negative cases. Previous studies have shown that ZOL was more efficacious in an estrogen-suppressed condition. However, the short-term application of ZOL in this study may not be sufficient to improve the outcome in postmenopausal patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-11

Title: Residual cancer burden (RCB) as a strong prognosis factor in breast cancer (BC) patients treated with neoadjuvant chemotherapy (NACT) based on carboplatin, doxorubicin and taxanes

de Juan Ferré A, Mayorga Fernández M, Alonso Bartolomé P, Azcarretazabal González-Ontaneda T, Muñoz Cacho P, Múgica Estébanez M, Anchuelo Latorre J, Mata Velasco E, Saíz Isa L and López Vega JM. Marqués Valdecilla University Hospital, Santander, Cantabria, Spain; Sierrallana Hospital, Torrelavega, Cantabria, Spain; Teaching Unit of Family and Community Medicine, Santander, Cantabria, Spain and Navarra Hospital, Pamplona, Navarra, Spain.

Body: Purpose: Many pathologic response systems have been used since the arrival of NACT. The RCB is a standardized method, but not universally implemented (*Symanns WF, et al. J Clin Oncol 2007*). The main objective is to apply the RCB and check its prognostic value in patients treated with platinum-based NACT with a long follow up.

Methods: Patients diagnosed and treated with platinum based NACT are analyzed. They receive 4 cycles of carboplatin AUC 6 and doxorubicin 50 mg/m², followed by either docetaxel 75 mg/m² (4 cycles) or weekly paclitaxel 80 mg/m² (8 doses), preoperatively. Pathological complete response (PCR) is defined as the absence of invasive tumor in the breast and axillary nodes, allowing the presence of DCIS. RCB system is applied and correlated with disease free survival (DFS) and overall survival (OS) according to Kaplan-Meier method; differences between curves with log rank test.

Results: 109 patients (110 tumors) are included from Mar-2004 Mar-2009. Characteristics of patients and tumors: Mean age: 50.9 years (range 28-78 years); Premenopausal: 69 (62.7%). Median tumor diameter: 35 mm (0-90 mm); histology: ductal 85 (77.3%). Stage: IIa 46 (41.4%); IIb 40 (36%); IIIa-11 (10%); IIIb 12 (10.9%); IIIc 1 (0.9%). Phenotypes: luminal A 18 (16.4%); luminal B 36 (32.7%); HER2 luminal B 22 (20%); HER2 13 (11.8%); triple negative 21 (19.1%). Responses: pCR: 17.3%; pCR and RCB-I: 37.3%. Responses according to phenotypes:

Table 1. Pathological response based on RCB and phenotypes

	Luminal A	Luminal B	Luminal B HER2	HER2	TN
pCR	0 (0%)	8 (22,2%)	1 (4,5%)	4 (30,8%)	6 (28,6%)
RCB-I	2 (11,1%)	6 (16,7%)	7 (31,8%)	5 (38,5%)	2 (9,5%)
RCB-II	13 (72,2%)	15 (41,7%)	12 (54,5%)	1 (7,7%)	6 (28,6%)
RCB-III	2 (11,1%)	6 (16,7%)	2 (9,1%)	2 (15,4%)	5 (23,8%)
Unknown	1 (5,6%)	1 (2,8%)	0 (0%)	1 (7,7%)	2 (9,5%)
Total	18 (100%)	36 (100%)	22 (100%)	13 (100%)	21 (100%)

. Surgery: lumpectomy 66 (60%). Follow up: DFS (median follow up 8,2 years (0,5-11,8)): 78,9%; OS (median follow up 8,6 years (0,8-11,41)). OS according to RCB: pCR: 94,7%; RCB-I: 86,4%; RCB-II: 76,6%; RCB-III: 58,8% (p 0,03).

Conclusion: RCB is a strong prognostic factor, here validated for a platinum based CT with a long follow up. Additional data on OS according RCB in different phenotypes will be presented at the meeting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-12

Title: Pertuzumab and trastuzumab in combination with weekly paclitaxel delivers high pCR rates with favourable toxicity profile in neo-adjuvant treatment of Her-2 positive breast cancer

Gupta N, Giblin E, Leagre C, Longmire-Cook S, Tigges T, Compton J, Dugan T, Liebross R, Givens S, Schmidt K, Paul R, Trivedi R, Smith B, Reeves D, Hanson J and Schmidt T. St. Vincent Cancer Care, St Vincent Hospital, Indianapolis, IN.

Body: Background: A combination of Pertuzumab (P) and trastuzumab (H) with chemotherapy is the standard of care for the neo-adjuvant treatment of locally advanced/inflammatory Her-2 +ve breast cancer. Neutropenia and diarrhea are the two common significant side-effects observed with the commonly used chemotherapy regimens. We evaluated the efficacy and safety of a combination of P and H with weekly paclitaxel (T) given in a neo-adjuvant fashion in Her-2 +ve patients.

Methods: Women with a tumor size >2 cm or LN positive or inflammatory breast Ca with Her-2 positivity by IHC or FISH were treated. The treatment regimen studied was; Pertuzumab on D-1 at a loading dose of 840 mg, followed by 420 mg every 3 weeks, Trastuzumab on D-1 at a dose of 8 mg/kg loading dose, followed by 6 mg/kg every 3 weeks, and Paclitaxel on D-1/8 at a dose of 80 mg/m². Six cycles were given at 3 week intervals. Herceptin was continued for a total of one year. Adjuvant radiation and hormonal treatments were given per the NCCN guidelines. Data on the pCR rate and safety profile were collected. LVEF was measured at baseline and every 6 weeks during neoadjuvant treatments.

Results: A total of 31 women were treated from Mar 2014 - Jan 2016. Median age was 43 years (31-80). Two pts had T-1 disease, 23 had T-2, 5 had T-3 and, 1 pt. had inflammatory breast ca. 16 pts had LN involvement. Receptor profile was: ER/PR +ve: 22 pts., ER+/PR-ve: 2 pts., ER/PR - ve: 7 pts.. All patients were able to complete the planned six cycles. Thirteen pts. achieved a pCR (41.9%). Five out of 7 ER/PR-ve patients (71.4%) had a pCR. Grade-3/4 neutropenia was seen in 3 patients (9.6%), febrile neutropenia was not observed in any pt. (0%). Grade-3/4 diarrhea was seen in 1 pt. (3.2%). Grade-3/4 neuropathy was seen in 2 pts. (6.4%). LVEF drop of more than 10% from baseline to less than 50% was seen in 1 patient (3.2%).

Conclusion: A combination of Pertuzumab, Trastuzumab and Paclitaxel (PHT) yields impressive pCR rates with minimal side-effects compared to the commonly used chemotherapy regimens. This combination could be explored further in larger trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-13

Title: Predictive and prognostic value of Ki-67 expression in triple-negative breast cancer before and after neoadjuvant chemotherapy with weekly paclitaxel plus carboplatin

Wang R-X, Chen S, Jin X and Shao Z-M. Shanghai Cancer Center, Shanghai, China.

Body: Background: Neoadjuvant chemotherapy (NCT) is one of the main treatment strategies for patients with locally advanced breast cancer. This study was designed to demonstrate the utility of a platinum-containing treatment regimen in the neoadjuvant setting and to identify the predictive or prognostic value of Ki-67 among patients with triple-negative breast cancer (TNBC) Methods: Data from 280 patients with stage II–III TNBC were collected. All patients underwent four cycles of NCT with paclitaxel (80 mg/m²) and carboplatin (AUC 2 mg*min/ml) on days 1, 8, and 15 of a 28-day cycle. Ki-67 values were evaluated in both biopsy and excision specimens. The clinical responses to NCT were evaluated based on MRI and ultrasound examinations in accordance with the response evaluation criteria in solid tumors (RECIST) version 1.1. The pathological evaluation of surgical specimens was conducted at by the Miller-Payne (MP) grading system. Logistic regression analysis was performed to identify independent predictors of pathological complete response (pCR). Survival analysis was performed to evaluate the prognostic value of various clinical and pathological variables.

Results: The overall pCR rate was 33.9%. Both the categorical and linear Ki-67 were independently correlated with pCR ($P < 0.001$). There were also statistically significant differences among Ki-67 categories with respect to clinical response ($P < 0.001$), Miller-Payne (MP) grades ($P < 0.001$), and node status ($P < 0.001$). A significant reduction of Ki-67 after NCT was most likely observed in patients with a relatively better response. Among 185 patients with a residual tumor after NCT, the tumor cell proliferation significantly decreased from a median Ki-67 value of 20.0% before chemotherapy to 15% after chemotherapy. The mean absolute reductions in Ki-67 were 29.4%, 8.5%, and -10.2% in patients with an ideal response (MP 5/4), a partial response (MP 3) and a poor response (MP2/1), respectively. In the multivariate model for non-pCR patients, Ki-67 reduction presented an independent prognostic value for relapse of disease (HR=0.986, 95% CI: 0.978-0.994; $P = 0.001$). Residual node involvement was also an independent predictor of patient outcome (HR=0.895, 95% CI: 0.355-2.259 for 1-3 nodes and HR=2.424, 95% CI: 1.048-5.608 for more than 4 nodes, using 0 nodes as a reference; $P = 0.002$). Better survival was more frequently observed in patients with a greater reduction in the Ki-67 value and fewer involved nodes.

Conclusions: The primary Ki-67 might help in further classifying TNBCs into subtypes with different responses to chemotherapy. The significant reduction of Ki-67 after treatment could indicate a favorable prognosis in non-pCR patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-14

Title: NOSTRA PRELIM: A non randomised pilot study designed to assess the ability of image guided core biopsies to detect residual disease in patients with early breast cancer who have received neoadjuvant chemotherapy to inform the design of a planned trial

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Body: BACKGROUND

Patients receiving neoadjuvant chemotherapy for breast cancer go on to have surgery regardless of their response. Women with HER-2 positive ER negative tumours (7.5% of all operable breast cancers in the UK) respond so well to neoadjuvant treatment with dual anti-HER2 therapy in combination with chemotherapy that pathological complete response rates of 80% can be achieved. This means that for patients receiving this treatment surgery is being performed to remove a cancer that isn't there in the large majority. A study is planned in the UK (NOSTRA) to assess if it is feasible to treat patients with HER-2 positive ER negative breast cancer who achieve a pCR after neoadjuvant chemotherapy with trastuzumab and pertuzumab with radiotherapy alone. The first phase of the trial will be a feasibility study commencing in 2017 where all patients will have image guided tumour bed biopsies post treatment and all patients will have surgery. If the group of patients where a pCR is achieved can be accurately identified then a phase III trial randomising to surgery and radiotherapy or radiotherapy alone is planned.

The NOSTRA PRELIM study reported here assessed the ability of post neoadjuvant chemotherapy tumour bed biopsies to detect residual disease to provide experience to inform the much larger NOSTRA feasibility trial biopsy protocol.

METHODS

23 consecutive patients with operable primary breast cancer scheduled for neoadjuvant chemotherapy were approached to take part in the study and 20 gave consent.

All 20 patients had a clip inserted into the tumour bed under ultrasound(USS) guidance at diagnosis as is standard procedure. The number of cores taken ranged from 2-6. The median number of biopsies was 4

Tumour size range at diagnosis was 15-61mm with USS. All received neo-adjuvant chemotherapy and those who were Her2 positive received neoadjuvant trastuzumab. At completion all patients had USS guided tumour bed biopsy. They then went on to have surgery, after which pathology was assessed and an RCB score calculated for each patient.

For this study patients all tumour types were included as non pCR outcomes were required to determine accuracy and inform changes to the biopsy protocol for future use. **RESULTS**

Only 2 patients in this study achieved a pCR

Residual disease was correctly identified in 16/20 patients.

Four patients had no tumour in their post treatment biopsies but had small residual invasive tumour at surgery. The size of residual disease in these patients ranged from 0.5 -9mm and all these patients had 3 core biopsies.

One patient had negative post treatment biopsies and a PCR of their invasive tumour. This patient had a diagnostic biopsy that confirmed separate area of DCIS several centimetres from the invasive component. This area did not undergo post treatment tumour bed biopsies (although both areas were clipped at diagnosis).

CONCLUSION

A protocol for biopsy in the upcoming NOSTRA feasibility study has been designed to both take more biopsies and sample a larger area of the tumour bed in order to minimise the false negative rate.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-15

Title: Lymph-vascular invasion in the absence of stromal invasion after neoadjuvant therapy: A rare pattern of residual carcinoma that lacks an AJCC/UICC T category

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Body: *Background:* Response to neoadjuvant chemotherapy in breast cancer serves as an important prognostic indicator, as patients whose estrogen receptor (ER) negative and/or HER2 positive cancers undergo a pathologic complete response (pCR) have an excellent outcome. Guidelines on classification of treatment response are available, but data is lacking for the rare event where lymph-vascular invasion (LVI) is the only residual disease in the breast. Only one study (Rabban JT et al, Am J Surg Pathol, 2009) has investigated the significance of this pattern of residual disease. These authors reported 6 patients with residual LVI in the breast in the absence of stromal invasion. Five of the patients also had residual disease in lymph nodes. Prognosis was poor with four of the patients dying in less than 4 years and an additional patient dying at 10 years. The aim of our study was to gather more data on this rare pattern of residual disease, with a focus on node negative cases. *Design:* We retrospectively identified from our pathology database all cases in which LVI was the only residual disease in the breast after neoadjuvant therapy. *Results:* A total of 16 cases were identified, yielding an incidence of 1.6% of all cancers undergoing neoadjuvant chemotherapy over a 10 year period. All patients were females with a mean age of 54 years (range 40-69 years). Eight cancers initially presented as a palpable breast mass, 4 as vague breast symptoms, 2 as inflammatory carcinoma and 1 was detected on screening mammogram. In twelve cases the axillary lymph nodes were either suspicious by imaging or proven positive by needle biopsy. The mean pre-neoadjuvant tumor size was 2.7cm (range 0.5-8.0cm). Six cancers were negative for hormone receptors and HER2, eight cancers were positive for HER2 and 4 cancers were positive for ER. After neoadjuvant chemotherapy, nine patients had no residual disease in lymph nodes. The remaining seven patients had residual disease in lymph nodes, although minimal in the majority. The mean follow-up was 65 months for the node negative group (range 9-125 months) and 44 months for the node positive group (range 7-102 months). One death occurred in each group (at 12 months and 67 months respectively), two patients are alive with metastatic disease in the node negative group and the remaining 12 patients are alive without disease. *Conclusions:* Pure residual LVI after neoadjuvant chemotherapy is a rare event. Our findings show that the outcome associated with residual LVI, with or without residual cancer in nodes, might not be as dismal as previously reported, although longer follow-up will be required. The more favorable outcome observed is in contrast to the single study previously published. Although the difference may be due to details in the type of chemotherapy or stage at presentation, it is difficult to compare the 2 groups of patients due to limited information about the earlier cases. More data is needed to draw conclusions on the prognostic significance of this type of residual disease. The lack of a current AJCC/UICC T category for this finding will make identification of these patients difficult in large databases.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-16

Title: Role of tumor microenvironment, as assessed by breast MRI background parenchymal enhancement (BPE), in modulating response to neoadjuvant chemotherapy in young women with localized breast cancer

Spring L, Rutledge G, Yala A, Haddad S, Specht M, Moy B, Barzilay R, Lehman C and Bardia A. Massachusetts General Hospital, Boston, MA and Massachusetts Institute of Technology, Cambridge, MA.

Body: Background:

Neoadjuvant chemotherapy (NACT) is generally established as a therapeutic option for selected high-risk patients with localized breast cancer, including triple negative breast cancer (TNBC). On a patient level, achievement of pathologic complete response (pCR) at the time of surgery is associated with improved long-term outcomes and is considered to be a surrogate marker. Response to NACT is a complex phenomenon dependent on both host and tumor characteristics. While tumor characteristics, such as receptors and tumor grade, have been well studied as predictors of pCR, host characteristics to predict pCR have been less well studied. Background parenchymal enhancement (BPE) is an imaging characteristic that reflects the normal enhancement of the fibroglandular tissue on breast MRI, and could potentially modulate response to NACT by influencing the tumor microenvironment and vasculature. The aim of this study was to explore the ability of baseline BPE to predict pCR in a cohort of young women with localized breast cancer.

Methods:

A retrospective chart review was conducted of women ages 40 and under with stage II-III breast cancer treated with NACT at our institution from 2004 – 2014. Demographic, clinical, and pathological variables were extracted from the medical records. The primary outcome was achievement of pCR, defined as ypT0/is ypN0, after NACT. BPE pattern in the contralateral breast was obtained from pre-treatment breast MRI reports if available and otherwise was retrospectively determined by a breast radiologist blinded to patient outcomes. BPE was dichotomized as low (minimum and mild) vs. high (moderate and marked). Logistic regression was used for statistical analysis.

Results:

A total of 69 patients ages 40 and under received NACT for localized breast cancer during the study period and had available pre-treatment breast MRI images. Median age at diagnosis was 36 (range 27-40). The majority of patients had grade 3 (65.2%), ER+/HER2- (60.9%) tumors while 24.6% had TNBC. Among pre-treatment breast MRIs, 42 (60.9%) patients had minimum or mild BPE and 27 (39.1%) patients had moderate or marked BPE. The overall pCR rate was 39%. After controlling for tumor grade, ER status, HER2 status, clinical stage, and type of NACT, high baseline BPE was associated with a trend towards higher odds of achieving pCR compared to low BPE (OR = 1.49, 95% CI 0.47–4.71), though statistical significance was not reached ($p = 0.50$). When stratified by ER status, the relationship was stronger among the ER+ subset (OR 1.8, $p = 0.49$) compared to the ER- subset (OR 1.3, $p = 0.78$).

Conclusions:

A statistically significant association between high baseline BPE and achievement of pCR was not found in this limited sample size, but a trend towards higher pCR rates, particularly with ER+ tumors, was seen. While tumor factors have traditionally been used to predict pCR, BPE is a readily available MRI imaging characteristic that reflects the tumor microenvironment and may be useful in building a model that incorporates tumor factors along with host factors to develop personalized NACT regimens for young women with breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-17

Title: Population based long term outcomes of pathologic complete response after neoadjuvant chemotherapy in stage I-III breast cancer: The British Columbia experience

Sun J, Gondara L, Diocee R, Speers C, Lohrisch C and Chia S. British Columbia Cancer Agency, Vancouver, BC, Canada.

Body: Background:

Neoadjuvant chemotherapy is a treatment option for breast cancer patients (pts) with locally advanced disease and for pts with operable breast cancer who desire breast conservation. Neoadjuvant therapy also allows for early evaluation of the effectiveness of systemic therapy. Pathologic complete response (pCR) has been shown in clinical trials to be associated with improved survival. The objective of this study was to determine if the outcomes demonstrated in clinical trials can be applied in the population based setting by comparing the outcomes of breast cancer pts who achieved pCR (no invasive disease in breast and nodes) vs. those that did not achieve a pCR.

Methods:

This is a retrospective cohort study of stage I-III invasive breast cancer pts treated with neoadjuvant chemotherapy from 2005 to 2010 in British Columbia. Cases were identified from the Breast Cancer Outcomes Unit database. Data was collected on demographics, tumor pathology, and type of treatment (chemotherapy, endocrine therapy, trastuzumab) and linked to standard clinical outcomes.

Results:

267 pts who met inclusion criteria were identified, of whom 5% had stage I, 33% Stage II and 59% Stage III breast cancer. Median follow up was 7.4 years. Overall 74 pts (28%) demonstrated a pCR and 193 pts did not. pCR pts had better 5-yr overall survival (OS) vs. non-pCR pts: 88% vs. 73% (HR 0.43, 95% CI 0.23-0.82, p=0.01). 5-yr disease free survival (DFS) was 84% in pCR pts vs. 70% in non-pCR pts (HR 0.45, 95% CI 0.24-0.83, p=0.01). Similarly, 5-yr breast cancer specific survival (BCSS) and distant disease free survival (DDFS) were significantly better in favor of the pCR cohort: HR 0.39 (95% CI 0.18-0.82, p=0.01) and HR 0.45 (95% CI 0.24-0.83, p=0.02) respectively. pCR pts were more likely to be HER2-positive and/or ER negative.

Conclusions:

Our population based results showed that early stage breast cancer pts who achieved pCR after neoadjuvant chemotherapy had better outcomes on all survival parameters compared to pts who did not achieve a pCR. This finding is consistent with results from neoadjuvant clinical trials and the FDA meta-analysis. These 'real world' results demonstrate that pCR can be used as a surrogate endpoint for survival outcomes even among non-trial pts.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-18

Title: The effect of participation in neoadjuvant clinical trials on outcomes in patients with early breast cancer

Brennan MB B, Wang D, Gass P, Hein A, Beckmann MW W and Fasching PA A. University of California, Los Angeles, Los Angeles, CA and University Hospital Erlangen, Comprehensive Cancer Center Erlangen-EMN, Erlangen, Germany.

Body: Pathological complete response (pCR) has been shown to demonstrate improved outcomes in breast cancer. Often novel treatments, such as taxanes or HER2 based therapies, trastuzumab or pertuzumab, have shown an increase of pCR with possible impact on the prognosis of breast cancer patients. These therapies are usually introduced into the patient population through clinical trials. Therefore, it can be hypothesized participation in neoadjuvant clinical trials might improve the pCR rate in early breast cancer patients. The aim of this study was to explore this association.

Methods: This is a retrospective study of consecutive breast cancer patients who were treated with a neoadjuvant chemotherapy at a single institution. Trial participation status was known for all patients. From 1995 to 2012 the institution participated in 10 different neoadjuvant chemotherapy trials. Outside those clinical trials, patients were treated according to national neoadjuvant therapy guidelines. Patients who took part in clinical trials were compared with patients who did not take part in clinical trials with appropriate univariate tests. Additionally, logistic regression was performed with pCR as the dependent variable and commonly known predictors of pCR (tumor size and molecular subtype) as independent variables. The model was adjusted for age at diagnosis, year of treatment and trial participation status. Furthermore, exploratory survival analyses were performed.

Results: A total of 281 patients were treated within a neoadjuvant clinical trial and 822 patients outside a clinical trial. There are significant differences in the patient populations with regards to age ($p=0.0009$) and tumor size (<0.0001). pCR rate in patients with trial participation was 21.4% and 19.0% in patients who did not take part in clinical trials. Multivariate logistic regression showed an OR of 1.393 (95%CI 0.937-2.071, $p=0.1012$). Exploratory survival analyses showed a better prognosis in patients taking part in clinical trials.

Conclusions: This study shows an increased chance of pCR in patients taking part in clinical trials; however the statistical test could not show statistical significance. Most likely the effect can be explained by trial participants having access to drugs which improved pCR rates, such as HER2 based therapies or taxanes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-19

Title: Evaluation of weekly paclitaxel plus carboplatin followed by anthracycline chemotherapy on the neoadjuvant treatment of patients with triple-negative breast cancer

Castrellon AB B, Velez M, Blaya M, Barnick S, Dumais K and LeCroy N. Memorial Cancer Institute, Hollywood, FL.

Body: Background

Triple negative breast cancer (TNBC) accounts for 15 to 20 % of the invasive breast cancers. Pathological complete response (pCR) in this subgroup of breast cancer is associated with improved long term event free survival. Results from previous studies indicate that the addition of carboplatinum (Cb) to standard neoadjuvant anthracycline-taxane chemotherapy results in an increase in pCR. One of the investigational arms of the CALGB 40603 tested paclitaxel 80 mg/m² once a week (wP) for 12 weeks with concurrent Cb (area under curve 6) once every 3 weeks for four cycles, followed by doxorubicin plus cyclophosphamide once every 2 weeks (ddAC) for four cycles. Although effective this regimen has been difficult to reproduce in the daily practice. The purpose of this study was to evaluate the effectiveness and tolerability of wP in combination with weekly Cb (wP+wCb) area under curve 2 (AUC=2) followed by anthracycline chemotherapy.

Methodology

The electronic medical record system was used to identify female patients 18 years of age or older with clinical stage I-III (TNBC) who received neoadjuvant chemotherapy of wP+wCb (AUC=2) before or after anthracycline chemotherapy between January 1, 2014 and March 1, 2016. The primary outcome was to evaluate the tolerability of fractionating carboplatin to weekly infusions in combination with weekly paclitaxel. The secondary outcomes included the pCR (no evidence of invasive tumor in the breast and axilla), Residual Cancer Burden (RCB), the number of cycles received in each chemotherapy regimen and frequency of chemotherapy related toxicities.

Results:

For the 32 eligible patients, median age: 51 years, Stage I: 6%, Stage II: 68%, Stage: III 26%, germline BRCA mutation: 10%, KI 67 > 75% : 72%. 93% of the patients received 11-12 cycles of wP+wCb and 83% received all 4 planned cycles of anthracycline chemotherapy. 83% of patients completed all planned therapy. pCR and RCB 0/1 rates were 60% (19/32) and 75% (24/32) respectively. RCB 2: 22% (7/32), RCB 3: 3% (1/32). 93% of the patients experienced grade 3 neutropenia during wP+wCb requiring GCSF, Grade 3 anemia was seen in 15 % (5/32) and Grade 3 thrombocytopenia was seen in 18 % (6/32).

Conclusion:

The combination of neoadjuvant chemotherapy with wP+wCb before or after anthracycline chemotherapy was well tolerated among patients with TNBC as demonstrated by the fact that most participants were able to receive all planned 12 cycles of wP+wCb and all 4 cycles of anthracycline chemotherapy. Complete pathologic response rates were comparable to historically seen. The findings support the continued use of this treatment modality in the general practice.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-20

Title: Impact of “immunohistochemistry-based molecular subtype” on chemo-sensitivity and survival in Hispanic breast cancer patients following neoadjuvant chemotherapy (NAC)

Gomez-Wolff R, Garcia H and Ossa C. Instituto de Cancerologia SA, Medellin, Antioquia, Colombia and Universidad de Antioquia, Medellin, Antioquia, Colombia.

Body: NAC it's the standard procedure when handling non-surgical tumors and inflammatory breast carcinomas, increases the possibility of conservative surgery and must be considered an evaluative tool in designing a therapeutic strategy. NAC improves Disease-Free Survival (DFS) and Overall Survival (OS) in patients achieving pathological complete response (pCR).

“Immunohistochemistry-Based Molecular Subtyping” (IMS) with ER, PR,HER2 and KI67 has shown good correlation with gene expression assays to identify Intrinsic Molecular Subtypes. The relationship between IMS, chemo sensitivity and survival is currently a matter of interest. Achieving pCR at the time of Surgery has been associated with a favorable prognosis.

Objectives:to investigate the pathological response to NAC and its correlation with IMS, as well as its correlation with DFS and OS in patients who have undergone NAC.

Methods: Women > 18 years old. treated with NAC and surgery at IDC (January 2009 to December 2013).Demographic, clinical en follow-up variables were obtained from de IDC patient's registry. Patient's vital status was traced trough phone calls.pCR was defined as ypT0 ypN0 (the absence of invasive cancer AND in situ cancer in the breast and axillary nodes). IDC's IRB approved the research project.

Results: A total of 592 patients fulfilled the study's inclusion criteria. The distribution by subtypes were: “Luminal A”: 150 (25.3%), “Luminal B/HER2 -”: 189 (31.9%), “Luminal B/HER2+”: 114 (19.3%), “HER2-enriched”: 45 (7.6%) and “Triple negative”: 94 (15.9%). The median follow-up was 51 months (interquartile range: 37-68).Pathological response to NAC was: complete pathological response (pCR) in 59 (10%) patients, partial pathological response in 426 (72.0%); stable disease in 82 (13.8%), and progression in 25 (4.2%) patients.In Multivariable Cox Regression Models, pCR is associated with a significant improvement in the DFS (HR 6.89; 95% CI: 2.53 to 18.75. p=0.01) and OS (HR 6.82; 95% CI 2.13 to 21.78. p=0.01). By

“Immunohistochemistry-based molecular subtype” the pCR is associated with a significantly better DFS for “Luminal B”, “Triple Negative” and “HER2-enriched”. For OS we found significantly association with pCR for “Triple negative” and “Luminal B” “Immunohistochemistry-based molecular subtype”. The “HER2-enriched”shown a trend to better OS if obtain pCR (p=0.05).

Conclusions: pCR is associated with an improved overall survival and disease-free survival rates in this group of Hispanics patients. In advanced stages, histological grade 3, Luminal B, Triple Negative and HER2+ enriched tumors the pCR is associated with a significantly better DFS .

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-21

Title: The association of higher tumor HER2 load with treatment response to neoadjuvant therapy in HER2 positive breast cancer patients

Lillemoe TJ J, Susnik B, Grimm E, Kang S-HL L, Swenson KK K, Krueger JL L, Finkelstein MJ J and Tsai ML L. Allina Health, Virginia Piper Cancer Institute, Minneapolis, MN.

Body: Background: Neoadjuvant therapy for HER2 positive invasive breast cancer is used to downstage tumor prior to surgery, objectively measure response, and evaluate novel therapies in clinical trials. Residual Cancer Burden (RCB) established by pathologic evaluation of post-treatment surgical specimens is a marker of tumor response to chemotherapy as well as predictor of recurrence-free survival in HER2 positive breast cancer. The relationship between the HER2 tumor load and the response to chemotherapy is not known.

Specific Aims: To evaluate RCB after neoadjuvant chemotherapy and HER2-targeted treatment for HER2-positive breast cancer, and to determine clinicopathologic factors associated with treatment response as represented by RCB.

Methods: This retrospective chart review included all HER2-positive breast cancer patients, stage I – III, receiving neoadjuvant chemotherapy and HER2-targeted therapy with post-treatment surgical resection at an Allina Health hospital from 2013-2016. Review of clinicopathologic variables included HER2/CEP17 ratio, HER2 absolute copies, ER/PgR status (using the H score calculation), patient age, baseline tumor size, and gross and microscopic pathology review of breast tissue specimens with RCB evaluation completed by breast pathologists. To compare factors related to response, chi-square with Monte-Carlo simulation was used to analyze dichotomous variables, and Mann Whitney U-tests were used for continuous variables.

Findings: The study included 97 patients. Upon surgical resection, complete pathologic response (RCB-0) was found in 47% (46/97 patients), and partial pathologic response (RCB-I) in 15% (15/97 patients). As compared to non-responders (RCB-2 and RCB-3) the complete and partial responders (RCB-0 and RCB-1) were associated with greater HER2/CEP17 ratios (10.2 vs. 6.5; $p=0.003$), and greater HER2 absolute copies (25 vs. 15.8; $p < 0.001$). Complete responders were associated with lower ER expression (H scores of 89.2 vs. 171.8; $p=0.005$) than non-responders, with RCB-1 responders associated with ER H-scores between RCB-0 and non-responders (H score=145.9). No significant differences were noted between responders and non-responders on age at diagnosis, pre-treatment tumor size, PgR expression, or the percentage of tumor infiltrating lymphocytes.

Conclusions: The majority of patients with HER2 positive tumors show considerable benefit with neoadjuvant chemotherapy and HER2-targeted treatment (63% in our study) based on RCB assessment. Predictors of response as measured by RCB include high HER2/CEP17 ratios, high absolute copies of HER2 signals by FISH, and lower ER expression.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-22

Title: Survival after adjuvant and neoadjuvant chemotherapy in elderly patients with triple negative breast cancer: A Cleveland Clinic experience

Mohapatra S, Seepana J, Haddad A, Spiro T and Daw H. Fairview Hospital/ Cleveland Clinic, Cleveland, OH and Moll Cancer Center, Fairview Hospital/ Cleveland Clinic, Cleveland, OH.

Body: Background:

Triple negative breast cancer (TNBC) comprises of approximately 15% of the total breast cancers. There is limited data on treatment outcome of these patients in elderly population. We report our experience with elderly patients with TNBC treated at our tertiary care centers.

Objectives and rationale:

1. Percentage of TNBC patients who received neoadjuvant chemotherapy (NACT) vs. Adjuvant chemotherapy (ACT).
2. Chemotherapeutic agents used for NACT and ACT.
3. Progression free survival (PFS) and overall survival (OS) after neoadjuvant CT and adjuvant CT.
4. Outcomes between elderly patients 65-74 years vs. 75 years and above.

Methods:

With IRB approval, the Cleveland Clinic's database was used to identify TNBC patients treated between 2000 to 2013. OFS from the diagnosis of TNBC was the primary end point. Cox proportional hazard models with stepwise variable selections were used for data analysis.

Results:

A total of 223 patients, divided in to two groups, were prospectively followed. The age group from 65-74 years consisted of "Group A" (N=140, median 69) and 75 or older formed "Group B" (N= 83, median 80, maximum 92). Eighty eight percent had ductal carcinoma. Clinically majority had stage I (50%) and stage II (33%) disease; 17% had stage III disease and most tumors were poorly differentiated (74%, grade 3). Slightly more than half the patients had lumpectomies (55%); and in only 13% of cases (25/190) were margins positive. Patients who received neoadjuvant (NACT), adjuvant chemotherapy (ACT) and radiation therapy (RT) were 65%, 53% and 38% respectively. For NACT primarily doxorubicin, cyclophosphamide and paclitaxel were used whereas for ACT it was docetaxel with cyclophosphamide. Group B had somewhat poorer performance status than younger patients ($p=.05$), received chemotherapy especially neoadjuvant therapy much less frequently ($p<.0001$). OS and PFS were significantly lower in Group B ($p=0.001$ and 0.007 respectively). This holds true even if one adjusts for performance status and use of chemotherapy ($p=0.04$ and 0.03 respectively).

Conclusion:

Among elderly patients with triple negative breast cancer, those above 75 years and older have poorer outcomes compared to those with age 65-74 years. However, this is a retrospective study and has limitations; prospective randomized studies are needed to confirm these findings.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-23

Title: Rapid germline *BRCA* screening for locally advanced breast cancer changes surgical procedure after neoadjuvant chemotherapy

Tunon de Lara C, Bonnet F, Debled M, Lafon D, Breton-Callu C, Rarouk-Simonet E, Fournier M, Petit A, Bubien V, Quenel-Tueux N, Lagarde P, Longy M, Macgrogan G and Sevenet N. Institut Bergonie, Bordeaux, France.

Body: Introduction

Neoadjuvant chemotherapy (NAC) is proposed in case of locally advanced breast cancer (LABC) to improve breast conservative treatment (BCT). In the case of germline *BRCA* mutated (gBRCAm) patients, risk-reducing prophylactic surgical strategies in France are mastectomy for pre-symptomatic. On the other hand, BCT is proposed to all patients after NAC according to clinical response, regardless their gBRCAm status. Moreover, in the case of *BRCA* mutation, local recurrence risk at 15 years is higher in the BCT group (23%) vs mastectomy (5%) (Pierce 2010). The aim of this retrospective one-institution analysis is to evaluate if the knowledge of gBRCAm status impact surgical decision.

Patients and methods

All patients who underwent *BRCA* genetic testing during NAC for ≥ 3 cm breast cancer between 2012 and 2015 were included. *BRCA* testing was decided with each patient based on age, familial history of breast or ovarian cancer and histological characteristics of the tumor. Rapid germline *BRCA* mutation screening was performed through targeted next generation sequencing with a 25-genes panel including full coding sequence of *BRCA1* & 2. Deleterious mutations were detected using MiSeq reporter and confirmed by Sanger sequencing before giving the results to the clinical geneticist, and finally used for the choice of surgical strategy. At the end of NAC (6 three-weeks cycles in our center), a shared-decision making for surgical procedure was performed, based on pre and post-NAC clinical and radiological features, and results of the genetic testing.

Results

A total of 25 patients (including three with bilateral cancer) were tested during NAC: mean age 38 years (26-64); mean clinical size 46 mm (20-130mm); histological types: triple negative (n=14), HER-2 positive (n=7), luminal (n=7). A germline *BRCA* mutation was detected in 10 patients (40%) : 8 *BRCA1* and 2 *BRCA2*, including 8 patients among the 14 patients with a Manchester score > 20 (6 *BRCA1* & 2 *BRCA2*). Two patients were secondarily excluded (one being metastatic and one died during NAC), one of them having a gBRCAm status. All the 23 patients evaluable for the surgical procedure after NAC could be eligible for a BCT. All the 9 patients with gBRCAm status choose mastectomy in the shared-decision making procedure while a BCT was performed in 12 of the 14 remaining patients without *BRCA* mutation.

Discussion

In this highly selected subgroup of patients, gBRCAm rate is higher (40%) than the usual rate for *BRCA* testing (17% in our center). Regarding the rationale for BCT or mastectomy procedure in LABC or pre-symptomatic gBRCAm patients, the duration of NAC allows rapid germline *BRCA* screening that looks very useful considering the high incidence of mutation we observed and the impact on surgical final decision. Furthermore, in the group of high Manchester score (>20), patients without *BRCA* mutation harbored incidental mutation, currently under analysis, especially on other genes involved in hereditary breast cancer, that could also be used as a compelling argument for mastectomy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-24

Title: Prognostic factors after neoadjuvant chemotherapy in breast cancer: Surgery type as a new prognostic factor

Fujihara M, Kin T, Yoshimura Y, Kajiwarra Y, Ito M and Ohtani S. Hiroshima City Hiroshima Citizens Hospital, Hiroshima, Japan.

Body: Background: Pathological complete response (pCR) after neoadjuvant chemotherapy (NAC) is not necessarily linked to long-term survival. Response to chemotherapy and outcomes after NAC differ among breast cancer subtypes, so we analyzed prognostic factors by subtype.

Methods: We retrospectively analyzed 451 patients treated with anthracycline and taxane-based NAC between 2007 and 2015. Trastuzumab was added for human epidermal growth factor receptor (HER)-2-positive breast cancer. pCR was defined as no residual invasive breast carcinoma; noninvasive residuals and infiltrated lymph nodes were allowed. In our institute, mastectomy was performed in patients in whom the breast could not be preserved, such as patients with large residual tumors and diffusely spread tumors throughout the breast after NAC. Kaplan–Meier and univariate and multivariate cox regression analyses were used to evaluate disease-free interval (DFI) and DFI prognostic values, respectively.

Results: Median follow-up was 43 months; median age was 56 (range, 23–88) years. The 3-year DFI and OS were 82.1% and 94.4%, respectively. In total, 85 patients had recurrence (18.8%) and 31 patients died (6.9%). Response rate (RR) was 93.4% (421/457). pCR rate was 26.2% (118/451) in all cases: 0% (0/82), luminal A; 10.9% (14/128), luminal B HER2(-); 43.1% (31/71), luminal B HER2(+); 59.4% (38/64), HER2; and 34% (36/106), triple negative (TN). For all subtypes, patients who achieved pCR had a non-significantly higher DFI.

Multivariate cox regression showed these associations with DFI: surgery type and Ki-67 > 30% for all cases and luminal B HER2(-); ypN (lymph node status after NAC), luminal B HER2(+);ypN and menopausal status, HER2; and age, surgery type, and clinical lymph node status (cN), TN.

Kaplan–Meier analysis showed that surgery type was strongly associated with DFI after NAC. Mastectomy patients had significantly poorer prognoses than partial mastectomy patients for all subtypes except HER2. For all cases, the median DFI in mastectomy patients was 73 months, but DFI was not reached in partial mastectomy patients ($p < 0.0001$). Compared with partial mastectomy patients, mastectomy patients had more advanced disease in terms of tumor size, lymph node status, and stage and showed lesser clinical and pathological responses to NAC and effects on ypN. Furthermore, first recurrences in mastectomy patients were often distant metastases, leading to poor prognosis.

Moreover, we analyzed the prognostic factors in 118 patients who achieved pCR. Univariate Cox regression analysis showed the association of the following with DFI: age (≤ 40 , > 40), cN, stage, surgery type, and ypN for all cases; decreasing Ki-67 values after NAC, luminal B HER2(-); clinical tumor size (cT), cN, surgery type, and luminal B HER2(+); ypN and HER2; age (≤ 40 , > 40), cN, stage, surgery type, and TN. In multivariate cox regression analysis, age (≤ 40 , > 40), surgery type, and ypN were independent predicting factors for all cases.

Conclusions: Prognostic factors after NAC differ among subtypes. Surgery type was strongly associated with outcomes after NAC, so it could be an independent prognostic factor.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-25

Title: Serum 25-hydroxyvitamin D levels and oncologic outcomes of breast cancer patients receiving neoadjuvant chemotherapy

Park S, Lee JS, Kim JH, Lim SM, Park HS and Kim SI. Yonsei University College of Medicine, Seoul, Republic of Korea and Yonjin Severance Hospital, Yonsei University College of Medicine, Yonjin, Republic of Korea.

Body: Background: According to prior studies, the role of serum vitamin D3 has been inconsistent and clinical implications of 25-hydroxyvitamin D (25-OHD) have been little studied in breast cancer patients receiving neoadjuvant chemotherapy (NCT).

Objectives: The aims of study were to investigate changes in 25-OHD levels before and after NCT and to determine the association of 25-OHD and oncologic outcomes including pathological complete response (pCR) in breast cancer patients.

Patients and Materials: From January 2010 to December 2013, serum 25-OHD levels at pre- and post-NCT were measured in 377 breast cancer patients. The association of serum 25-OHD levels with clinicopathological data including breast cancer subtypes, pCR and survival were retrospectively analysed. Delta 25-OHD was calculated as serum 25-OHD levels before minus after NCT.

Results: Mean age of study population was 48.7 years and mean follow-up periods were 35.9 months. Mean baseline serum 25-OHD concentration was 14.60 ng/mL (standard deviation, 7.44) and more than 80% of patients showed insufficient 25-OHD levels. The mean 25-OHD at post-NCT was 12.16 ng/mL (standard deviation, 6.87). There was a significant decrease in serum 25-OHD after NACT ($p < 0.001$). The pCR rates were 25.7% among study cohort. However, 25-OHD levels at baseline and post-NCT were not related to pCR and survival outcomes. No associations were found between pCR and delta 25-OHD. According to stratification by breast cancer subtypes, however, patients with ≥ -2.67 (median value) of delta 25-OHD revealed a trend of higher achievement of pCR and better survival in luminal A subtype. No associations were found among the other subtypes.

Conclusions: Many Korean breast cancer patients showed insufficient serum 25-OHD levels at diagnosis of malignancy and a significant decrease in serum vitamin D3 after NACT was observed. No significant association of 25-OHD with pCR and survival was found. Therefore, correction or maintenance of appropriate serum 25-OHD levels should be focused for bone health as comprehensive management of breast cancer during NCT. In addition, possible oncological aspects of 25-OHD should be further researched individually considering breast subtypes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-26

Title: National trends in neoadjuvant therapy for breast cancer

Schnabel F, Schwartz S, Hochman T, Chun J and Goldberg J. New York University Langone Medical Center, New York, NY and New York University Langone Medical Center, New York, NY.

Body: Purpose:Neoadjuvant therapy has been widely integrated in the treatment of locally advanced breast cancer. Over time, this strategy has been extended to include patients with earlier stage disease to allow for assessment of in vivo response to treatment. The aim of this study was to describe the national trends in neoadjuvant therapy for all invasive breast cancers with a particular focus on triple negative disease and HER2 status.

Methods: The National Cancer Database (NCDB), an oncology outcomes database that collects data from more than 1500 Commission on Cancer (CoC) accredited cancer programs, was queried for all women diagnosed with invasive breast cancer from 2006-2013. Patients with unknown systemic therapy sequence were excluded. Women were classified by whether or not they received neoadjuvant systemic, chemo and/or endocrine, therapy.

Results: We identified 1,221,976 cases that were eligible for this analysis. Of these, 29.7% were HER2 negative, 18.4% were classified as triple negative, and 8.9% received neoadjuvant systemic therapy. The percentage of patients receiving neoadjuvant therapy increased from 7.5% in 2006 to 9.8% in 2012 with a slight decrease to 9.5% in 2013. This increase in the use of neoadjuvant therapy over the time period was statistically significant ($p < 0.0001$).

Number and percent of patients who received/did not receive neoadjuvant therapy by year

2006 (N=136117)	2007 (N=143033)	2008 (N=148888)	2009 (N=154713)	2010 (N=154040)	2011 (N=162333)	2012 (N=163395)	2013 (N=159457)
No Neoadjuvant Therapy 125908 (92.5)	131559 (91.98)	136593 (91.74)	141364 (91.37)	139459 (90.53)	146500 (90.25)	147401 (90.21)	144306 (90.5)
Neoadjuvant Therapy 10209 (7.5)	11474 (8.02)	12295 (8.26)	13349 (8.63)	14581 (9.47)	15833 (9.75)	15994 (9.79)	15151 (9.5)

There was a small increase in the percent of patients with HER2 positive status who received neoadjuvant therapy, from 5.7% in 2006 to 6.5% in 2013. During this time period, there was a 9% increase in the percent of triple negative patients who received neoadjuvant therapy (13.1% in 2006 to 22.1% in 2013).

Conclusions: Over the time period from 2006-2013, there has been an apparent increase in the percentage of patients who received neoadjuvant therapy. This trend is accompanied by increases in the percentage of TNBC patients and in Her2 positive patients who received neoadjuvant therapy. Other factors and the joint effects of these factors on the observed increase in the use of neoadjuvant therapy are under evaluation to elucidate the basis for this observation in the NCDB data.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-27

Title: Variation in the use of neoadjuvant chemotherapy for breast cancer in the Netherlands. A lack of consensus

Spronk PER ER, Bommel van ACM CM, Siesling S, Vrancken Peeters M-JT T and Smorenburg CM M. Leiden University Medical Centre, Leiden, Netherlands; Comprehensive Cancer Centre the Netherlands (IKNL), Utrecht, Netherlands; Netherlands Cancer Institute / Antoni van Leeuwenhoek, Amsterdam, Netherlands and Institute for Biomedical Science and Technical Medicine, University of Twente, Enschede, Netherlands.

Body: Introduction

Neoadjuvant chemotherapy (NAC) is an important initial strategy in the management of stage III locally advanced breast cancer (LABC) as being advised in our national guidelines. Furthermore, NAC is increasingly being used in stage II BC for downsizing the tumour in order to enable breast conservation therapy (BST). The aim of this study was to examine which patient-, tumour- and hospital related factors influence its use in clinical daily practice. In addition, we investigated the preferences of the surgical- and medical- oncologist as well as the patients'.

Methods

- (1) All operated women [aged 18-70] from January 2011 to September 2015 were selected from the multidisciplinary NABON Breast Cancer Audit (NBCA). Multivariable logistic regression was used to assess significant independent predictors of NAC use.
- (2) Both specialists and ex-patients were provided with a survey about decision-making.

Results

Overall, 21% of stage II (N=18011) and 70% of stage III patients (N=1715) received NAC. Factors associated with NAC utilization included young age, a diagnostic MRI, histology, large tumour size, more advanced nodal disease, hormone-receptor negative tumours and participation in neoadjuvant trials. After case mix correction variation between hospitals remained (range 10% – 90%). Evidenced by additional survey research, reasons for indication of NAC vary widely among specialists (response rate of 24%; N=100). From the patient survey (response rate of 52%; N=390) it appears that in patients where chemotherapy is recommended, 50% was not informed about the option of NAC.

Conclusion

There is considerable variation in the use of NAC for BC in the Netherlands. Although various patient, tumor and institutional factors are associated with the use of NAC, these can only explain part of the observed variation in treatment patterns between hospitals. Survey research shows a need for uniformity of selection criteria in the use of NAC between specialists. Also, the information necessary for patients to decide on which treatment option they prefer seems far from complete in clinical practice.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-28

Title: A single-institution clinical experience with neoadjuvant docetaxel/carboplatin/trastuzumab/pertuzumab (TCHP): A safety and efficacy analysis

Tariq R, Ajaz B, Shah N, Mamounas E and Moroosse R. UF Health Cancer Center- Orlando Health, Orlando, FL.

Body: Background: Pertuzumab, a monoclonal antibody targeting subdomain II of HER2 and blocking dimerization, was approved by the FDA in 2013 for use in combination with trastuzumab and docetaxel as neoadjuvant therapy for pts with HER2+, locally advanced, inflammatory or early-stage breast cancer (>2cm and/or node-positive). This accelerated approval was based on results from the NeoSphere and the TRYPHAENA trials. In NeoSphere, pathologic complete response in the breast and nodes [pCR] was 39.3% after 4 cycles of neoadjuvant pertuzumab/trastuzumab/docetaxel. In TRYPHAENA, pCR was 63.6% among 76 patients treated with 6 cycles of neoadjuvant TCHP (47.5% in pts with ER+and/or PR+/HER2+ tumors and 81.1% in those with ER-/PR-/HER2+ tumors). Aside from TRYPHAENA, we have limited information on clinical outcomes, with neoadjuvant TCHP. Here we report our institutional experience at UF Health Cancer Center, Orlando (UFHCC) with Neoadjuvant TCHP in patients with operable or locally advanced breast cancer.

Patients and Methods: After IRB approval, electronic medical record search was performed in order to identify HER2+ patients with tumors T2-T4/N0-3 or Tany/N1-3, treated with neoadjuvant TCHP between 10/13 and 5/16. Information from chart review included patient and tumor characteristics at the time of diagnosis, details of neoadjuvant chemotherapy plus anti-HER2 therapy, clinical, radiologic and pathologic assessment of tumor response to neoadjuvant TCHP, type of breast and axillary nodal surgery, surgical outcomes as well as disease outcomes.

Results: 76 patients (75 female, 1 male) met the inclusion criteria; median age: 52 yrs; 83% of pts presented with clinical stage II and 17% with clinical stage III; 62% were ER+ and/or PR+ and 38% were ER-/PR-negative. 49 patients received all planned 6 cycles without dose reduction. The remaining 27 patients required dose reduction due to rash, diarrhea, nausea, vomiting, neuropathy or neutropenia; 5 patients requested dose reduction due to poor quality of life and fatigue; 2 patients required dose delay due to asymptomatic cardio-toxicity with $\geq 10\%$ drop in EF. None had symptomatic CHF; 37% of patients underwent breast conserving surgery, 7% unilateral mastectomy and 55% bilateral mastectomy. Surgical lymph node assessment was performed after neoadjuvant chemotherapy and included sentinel lymph node biopsy (SLNB) in 74%, axillary dissection (ALND) in 8% or both in 18% of pts. Overall pCR rate (ypT0/is, ypN0) was 63.2%. pCR rate was 53.1% in pts with ER+ and/or PR+ tumors and 79.3% in those with ER-/PR- tumors. pCR by stage was 61% for Stage IIA, 65% for Stage IIB, 67% for Stage IIIA and 71% for Stage IIIC. Toxicity profile was consistent with what has been observed in the TRYPHAENA trial with fatigue, nausea, vomiting and neuropathy being the more commonly noted grade 3/4 toxicities. With median follow up of 18 months, all patients are disease-free with no documented recurrences observed.

Conclusion: Our clinical experience with neoadjuvant TCHP confirms the efficacy and safety data from the TRYPHAENA trial in a single-institution, tertiary care center setting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-29

Title: Nanoparticle albumin-bound paclitaxel as neoadjuvant chemotherapy of breast cancer: A meta-analysis

Zong Y, Wu J and Shen K. Comprehensive Breast Health Center, Shanghai Ruijin Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China.

Body: Background: Nanoparticle albumin-bound paclitaxel (nab-Paclitaxel), a novel solvent-free taxane-based regimen, was hypothesized to have enhanced drug transport to tumors, shorter infusion schedules and no need for premedication. The value of nab-Paclitaxel in neoadjuvant systemic therapy (NST) for breast cancer remains uncertain. We performed a meta-analysis to assess efficacy and toxicity of nab-Paclitaxel compared to conventional taxane regimens (paclitaxel, docetaxel) within randomized clinical trials.

Methods: A systematic search was performed using the medical subject heading (MeSH) terms "breast neoplasms", as well as (1) breast cancer; AND (2) nab-Paclitaxel OR nanoparticle paclitaxel; AND (3) neoadjuvant OR preoperative OR primary systemic in both Pubmed databases and proceedings of oncologic meetings including ASCO, ESMO and SABCS. Pooled rates of pathological complete response (pCR), odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using fixed-effect or random-effect model to determine the effect of neoadjuvant nab-paclitaxel.

Results: Twenty-one studies with 2357 patients were included, 3 of which (GeparSepto^[1], ETNA^[2], Showa trial^[3]) were randomized clinical trials. The aggregate pCR rate (ypT0/is ypN0) was 32% (95% CI 25-38%) in unselected breast cancer patients and was 14%(95% CI 11-17%), 41%(95% CI 38-45%), 54%(95% CI 43-66%) in hormone receptor-positive/human epidermal growth factor receptor 2-negative (HR+/HER2-), triple negative breast cancer (TNBC), HER2+ patients, respectively. Within the HER2+ population, pCR rate was 61%(95% CI 47-74%) for HR- and 40%(95% CI 28-52%) for HR+ tumors. Regarding randomized clinical trials, the probability of achieving pCR was significantly higher in the nab-paclitaxel group than in the conventional taxanes group (OR=1.383, 95%CI 1.141-1.676, p=0.001). A funnel plot of the effect size for each randomized trial against the precision showed no asymmetry, which indicating no potential publication bias. In the safety analysis (GeparSepto^[1], ETNA^[2]), hematological toxic effects were generally equivalent in nab-paclitaxel and paclitaxel group. For non-hematological toxic effects, all grades and grade ≥ 3 peripheral sensory neuropathy occurred more frequently with nab-paclitaxel compared to paclitaxel (all grades, OR=2.090, 95%CI 1.016-4.302, p=0.045; grade ≥ 3, OR=3.766, 95%CI 2.324-6.100, p<0.001). Hypersensitivity was more common with paclitaxel than nab-paclitaxel at any grade and grade ≥ 3. Other non-hematological toxic effects did not significantly differ between two groups.

Conclusion: nab-Paclitaxel is an effective antitumor drug in NST of breast cancer, especially for TNBC and HER2+ tumors, in terms of pCR. Exchange of nab-Paclitaxel for conventional taxanes could significantly improve pCR rate with reasonable toxicities.

Clinical trial information: 1. Lancet Oncol.2016,17:345-56.; 2. J Clin Oncol.2016,34(suppl; abstract 502).; 3. J Clin Oncol.2015,33(suppl; abstract 136).

2016 San Antonio Breast Cancer Symposium

Publication Number: P5-16-30

Title: Feasibility trial for identification of patients for eliminating breast cancer surgery following neoadjuvant systemic therapy

Kuerer HM M, Rauch GM M, Krishnamurthy S, Adrada BE E, Caudle AS S, DeSnyder SM M, Santiago L, Lucci A, Hobbs BP P, Gilcrease M, Hwang R, Candelaria RP P, Chavez Mac-Gregor M, Arribas E, Moseley T, Teshome M, Miggins MV V, Smith BD D, Valero V, Hunt KK K and Yang WT T. University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Contemporary improved neoadjuvant systemic therapy (NST) for breast cancer may result in a pathologic complete response (pCR) in up to 60% of patients (pts) yet imaging alone has a poor negative predictive value to determine which pts might be spared surgery. This study was designed to evaluate the hypothesis that percutaneous image guided biopsy after NST can accurately identify patients who may forgo surgery.

Methods: Prospective single-center IRB approved study of 34 pts with clinical T1-3 N0-3 triple-negative (TN, n=23) or HER2-positive (n=11) invasive ductal cancer who received standard NST and consented for ultrasound/mammography guided vacuum-assisted core biopsy (VACB) and fine-needle aspiration (FNA) biopsy prior to standard surgery. Main outcome measures included accuracy, false-negative rate (FNR), and negative predictive value of image guided biopsy in predicting residual disease after NST. Breast pCR was defined as no residual DCIS or invasive disease. Final biopsy showing atypia and/or suspicion of residual disease was recorded as positive.

Results: Median initial maximum tumor size based on imaging and physical exam was 3 cm (1.2-7 cm) and 47.1% had FNA/core biopsy proved nodal metastases. Final median maximum residual tumor size after NST was 0.9 cm (0-4.2 cm) with 94.1% having no palpable abnormality. Median number of VACB (9G) removed following NST was 10 (4-14) and was performed by stereotactic (67.6%) or ultrasound (32.4%) guidance. Overall, a breast pCR occurred in 18 (52.9%) of pts and breast pathologic response was concordant with nodal pathologic response in 33 (97%) of pts (1 pt with a breast pCR had 1/15 nodes with metastases). Overall, VACB combined with FNA following NST had a 100% (95% CI 89.7-100) accuracy, 0% FNR (95% CI 0-20.6), and 100% (95% CI 81.5-100) negative predictive value for determination of residual breast disease. Grade 1 adverse events which resolved from biopsy (bleeding, hematoma, bruising) occurred in 6 pts (17.6%).

Conclusions: High rates of pCR among pts with TN/HER2-positive breast cancer receiving NST occur in a significant proportion of pts. The use of image guided VACB/FNA can identify pts after NST where significant residual disease is unlikely. Based on these results, an IRB approved clinical trial will shortly commence for pts with T1-2 TN/HER2-positive breast cancer with documented image guided biopsy proved pCR after NST to be followed by standard definitive whole-breast radiotherapy without surgery.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-01

Title: The SIRT2 deacetylase stabilizes Slug to control malignancy of basal-like breast cancer

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Body: Overabundance of Slug protein is common in human cancer and represents an important determinant underlying the aggressiveness and poor survival of basal-like breast cancer (BLBC). Despite its importance, this transcription factor is rarely mutated in BLBC, and the mechanism by which it becomes deregulated in cancer remains unknown. Here we report that Slug undergoes acetylation-dependent protein degradation and identify the deacetylase SIRT2 as a key mediator of this post-translational mechanism. SIRT2 inhibition rapidly destabilizes Slug, whereas SIRT2 overexpression extends Slug stability. We show that SIRT2 deacetylates Slug protein at lysine residue K116 to prevent Slug degradation. Interestingly, SIRT2 is frequently amplified and highly expressed in BLBC. Genetic depletion and pharmacological inactivation of SIRT2 in BLBC cells reverses Slug stability, causes the loss of clinically relevant pathological features of BLBC, and inhibits tumor growth. Collectively, these findings unravel a novel molecular interplay between SIRT2 amplification, Slug perdurance and the BLBC phenotype. As such, targeting SIRT2 may be a rational strategy for diminishing Slug abundance and its associated malignant traits in BLBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-02

Title: Integration of whole-genome sequencing and functional screening to identify breast cancer lung metastatic drivers

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Body: Purpose: Changes in phenotype and genotype of breast cancers during the metastatic process remain to be elucidated. Here, we aim to characterize genetic alterations and potential drivers underlying breast cancer lung metastases using whole-genome sequencing. To discern driver from passenger genes, we developed an *in vivo* gain-of-function screening for metastases-related copy number variation (mCNV) genes using Gene-barcoding library.

Methods: We retrospectively enrolled 7 metastatic breast cancer (MBC) patients receiving surgical removal of lung metastases. Matched primary and metastasis tumors were collected. In total, whole-genome sequencing was performed on either frozen or formalin-fixed paraffin-embedded samples with 50- and 30-fold coverage for tumoral and normal tissues. A gain-of-function screening was performed in lung metastasis model using MDA-MB-231 cells (multiplicity of infection= 0.2) with a retrovirus-based human open reading frames (ORF) pooled library. Each gene was labeled with 3 unique barcodes. The lung metastases enriched genes were identified using polymerase chain reaction and next-generation sequencing (NGS).

Results: For 7 patients enrolled in this study, MBC1 was triple-negative breast cancer (TNBC), MBC2~5 were hormone receptor (HR) positive and human epidermal growth factor receptor 2 (HER2) negative, and MBC6~7 was HR-/HER2+. Median age at primary diagnosis was 50 years (range, 28-70 years). Median disease-free interval was 36.4 months (range, 19.7 -62.3 months). Most patients received anthracycline/taxane based adjuvant chemotherapy, except for two HR+ patients. All HR+/HER2- patients received adjuvant endocrine therapy, and all HER2+ patients received adjuvant trastuzumab therapy. Patient MBC1 had a high frequency of novel mutations in the lung metastases, including TP53, CYP19A1, PCDH9, NCAM2, MAP2K7 and MAPK4. In contrast, the somatic mutation profiles of MBC2~7 show high concordance between primary tumor and metastases. CNV discordances were common in TNBC and HR+/HER2- cases. Meanwhile, changes in CNV were rarely observed in HR-/HER2+. In TNBC and HR+ patients, we identified a series of copy number gain/amplification regions involving chromosome 3q25~26, 3q28~29, 8p11, 8q13, and 8q21~24 in lung metastasis compared with primary tumor. Recurrent copy number gain encoding genes (frequency $\geq 3/5$) were selected as metastases-related copy number variation (mCNV) genes, including PLSCR1, PTX3, ARL14, WHSC1L1, FGFR1, ADAM9, OXR1, EBAG9, TNFRSF11B etc. We therefore established a gene-barcoding ORF library for 65 mCNV encoding genes for the pooled screening in lung metastasis model. The readout of gain-of-function screening shows that PTX3, FGF12 and WDR49 are enriched in lung metastases.

Conclusion: Whole-genome copy number analysis suggested CNVs discordance between primary breast cancer and lung metastasis, especially in TNBC and HR+/HER2- disease. PTX3, FGF12 and WDR49 may play pivotal roles in driving lung metastasis of breast cancer. An *in vivo* gene-barcoding library screening enabled identify copy number gain/amplification genes likely contribute to cancer metastasis, providing a robust method to explore the phenotype-genotype correlation in cancer evolution.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-03

Title: N-terminus Slit2 suppresses breast cancer metastasis by inhibiting tumor associated macrophages

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Body: Distant metastasis is a major cause of breast cancer related death. Recent insights suggest that stromal cells including macrophages present in tumor microenvironment plays crucial role to enhance the metastatic potential of breast cancer cells. Primarily discovered as neuronal guidance cue, Slit2 has recently been emerged as an important tumor suppressor gene. Slit2 is reported to be genetically mutated and highly methylated in various cancers including breast cancer. Slit2 possess a proteolytic site and generates N-terminus and C-terminus fragments. We have previously shown that the N-terminus Slit2 (N-Slit2) contains biological activity and suppresses tumor growth. However, its effect on lung specific metastasis and tumor microenvironment is not studied yet. To analyze the effect of Slit2 on breast cancer growth and metastasis, we used MVT-1 cells derived orthotopic syngenic preclinical mammary tumor model. We observed that the Intra-tumoral injection of Adeno-virus particles overexpressing NSlit2 (Adeno-Slit2) significantly inhibited MVT-1 implanted tumor growth and lung metastasis compared to Adeno-virus particles expressing control vector (Adeno-Null). We further validated anti-metastatic activity of Slit2 using human breast cancer cell line MDA-MB-231 overexpressing N-Slit2 (231-Slit2) or control vector (231-Null) implanted in Nude/SCID/Gamma (NSG). The 231-Slit2 derived xenografts grow slower than 231-Null. Interestingly, the metastasis of 231-Slit2 to lung significantly reduced compared to 231-Null. To analyze the cellular events involved, we analyzed tumors for immune cell recruitment and observed less number of CD11b/F4/80/CD206 positive tumor associated macrophages (TAMs) in 231-Slit2 tumors compared to 231-Null. To further explore the underlying molecular mechanism, we analyzed the genes modulated by slit2 overexpression in MDA-MB-231 cells and observed decreased IL6 expression, which was further validated by protein array. In vitro assays depicted that the IL6 up-regulates various TAM markers and helps macrophage to convert to TAMs. We also analyzed Slit2 expression in human breast cancer TMA and an inverse correlation of Slit2 expression was observed with the incidence of breast cancer and distant metastasis. This study highlights the ability of Slit2 to prevent macrophage alternative activation to TAMs, thereby restrict tumor growth and lung metastasis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-04

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-05

Title: Novel cytotoxic RNA aptamers that distinguish between metastasis-prone and indolent breast and prostate cancers

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Body: Background: Prostate and breast cancers are, respectively, the most common malignancies diagnosed in men and women worldwide. These cancers develop in different organs but have significant biological similarities: both are typically hormone-dependent, and both require early detection and treatment, as metastatic disease is incurable. At the same time, early stage tumors are often over-treated. Better markers for tumor aggressiveness would help to optimize treatment strategies in both breast and prostate cancer.

Objective: Develop high affinity nucleic acid oligomers (aptamers) that can distinguish between indolent tumors that will remain organ-confined and those with heightened potential to metastasize.

Methods: We performed subtractive RNA Cell-SELEX to select for surface ligands specific to aggressive tumors, using as a positive selector the highly metastasis-competent LN3 subclone of prostate cancer cell line LNCaP, and as negative selectors parental LNCaP and a non-metastasizing subclone, Pro5. . The RNA aptamer pool was PCR amplified from a 40-mer random nucleotide cDNA library with appropriate flanking sequences, and transcribed *in vitro*. After 11 SELEX cycles, aptamer pools from cycles 0, 4, 9, and 11 were subjected to high-throughput sequencing. Eight aptamers, representing 4 sequence families, were chosen for further study. Representative relevant and irrelevant aptamers were labeled with Cy3 and used to stain LNCaP-LN3 and LNCaP-Pro5 in culture and as xenografts in NOD-SCID-gamma mice. Additional cell and tumor lines from both breast and prostate cancer were used for validation.

Results: Two aptamers bound avidly to the surface of the aggressive LNCaP-LN3 subclone, both in culture and in fixed xenograft tumors, but not to the indolent Pro5 subclone. Aptamer binding led to rapid and specific cytotoxicity *in vitro* but had no effect on other cell lines to which the aptamer did not bind. The same aptamers showed similar high specificity for multiple other metastasis-competent cancer cells, including the prostate adenocarcinoma PC-3 and PC-3ML subclones, breast cancer cell lines MDA-MB436 and MDA-MB231, and the primary dissociated breast tumor DT28 , while exhibiting no detectable binding to the non-metastasizing MCF-7 breast cancer cell line and DT22 primary dissociated breast tumor cells, and the non-tumorigenic prostate epithelial cell line RPWE-1.

Conclusion: We identified RNA aptamers that specifically bind to metastasis-prone prostate cancer and breast cancer cell surface targets, and exert cell-specific toxicity dependent upon aptamer binding. While the target(s) remain to be identified, we propose that these aptamers may discriminate between progressive and indolent breast and prostate cancers, and may have substantial promise as anticancer agents either alone or suitably liganded to toxic moieties.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-06

Title: Glutamine metabolism drives breast cancer invasion by providing a source of extracellular glutamate to activate the GRM3 metabotropic glutamate receptor

Macpherson IR R, Dornier E, Rabas N, Rainero E and Norman JC C. University of Glasgow, Glasgow, United Kingdom; CRUK Beatson Institute, Glasgow, United Kingdom and University of Sheffield, Sheffield, United Kingdom.

Body: Glutamine metabolism is well-established to contribute to cancer cell growth and proliferation by providing a source of nitrogen for nucleotide and amino acid biosynthesis as well as TCA cycle intermediates. There is also accumulating evidence that glutamine metabolism may contribute to metastasis although mechanistic links to tumour cell migration and invasion remain unclear. We have generated a number of highly invasive primary cell lines from the polyoma middle-T genetically engineered mouse model of breast cancer (MMTV-PyMT) and found that withdrawal of glutamine from these cells reduces not only their proliferation, but also their invasive migration into 'stroma-like' preparations of fibroblast-derived extracellular matrix. Our metabolomic analyses indicate that invasive MMTV-PyMT cells actively secrete glutamate, a product of glutamine metabolism, into the extracellular milieu. Moreover, addition of glutamate is sufficient to restore invasiveness (but not cell growth or proliferation) to glutamine-starved MMTV-PyMT cells. We have pursued these findings by investigating the role played by plasma membrane receptors for glutamate in cell migration and invasion in PyMT cells and in MDA-MB-231 triple negative breast cancer cells. We provide evidence that glutamate generated within the cell by deamidation of glutamine leaves the cell via the xCT antiporter to activate the GRM3 metabotropic glutamate receptor at the cell surface. This, in turn, suppresses adenylate cyclase activity to prevent protein kinase A activation and to drive an invasive programme. Indeed, knocking out GRM3 with CRISPR technology or inhibition using a selective GRM3 antagonist (LY341495) is sufficient to oppose invasiveness without compromising proliferation. Conversely, a specific GRM3 agonist (LY354740) drives invasiveness without increasing proliferation. Consistently, treatment with LY341495 was sufficient to abrogate lung colonisation following tail vein injection whilst tumour growth after orthotopic injection was unaffected. Our results provide a mechanistic link between glutamine metabolism and invasion and identify GRM3 as a potential therapeutic target in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-07

Title: Mitochondria-nuclear communication regulates epithelial-mesenchymal transition and metastasis in triple negative breast cancer

Park JH, Jung KH, Sirupangi T, Vithayathil S, Jin F, Putluri V, Piyarathna DWB, Yotnda P, Bhat VB B, Sreekumar A, Lewis MT T, Coarfa C, Putluri N, Creighton CJ J, Wong L-JC C and Kaiparettu BA Abraham. Baylor College of Medicine, Houston, TX; Baylor College of Medicine, Houston, TX; Dan L. Duncan Cancer Center-Biostatistics, Baylor College of Medicine, Houston, TX; Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX and Agilent Technologies, Wilmington, DE.

Body: For triple negative breast cancer (TNBC), the driver pathways are still poorly understood. Advances in cancer metabolism research over the last decade have enhanced and modified our understanding on Warburg effect. It is now known that mitochondria in tumors are not always defective in their ability to carry out oxidative phosphorylation. Instead, in proliferating cells, mitochondrial energy pathways are reprogrammed to meet the challenges of macromolecular synthesis and to escape from apoptosis. Tumor initiating cells (TICs) maintain cancer stem cell properties and are known to play significant role in TNBC metastasis. Mitochondrial retrograde regulation (MRR) is a bidirectional communication between mitochondria and nucleus. MRR is triggered by mitochondrial functional demands and it responds in a continuous manner to change metabolic needs of the cell. Using transmitochondrial cybrid (cybrid) technology, we generated different cybrid models under common nuclear backgrounds of benign breast epithelium or TNBC. Mitochondria from cells with different cancer potential such as benign breast epithelium, moderately metastatic and highly metastatic breast cancer cell lines were studied under the common nuclear background to understand MRR-regulated TIC properties and cancer pathways. Using genomic, metabolomic, and proteomic approaches, we confirmed the significance of mitochondrial character in the regulation of epithelial mesenchymal transition (EMT), TIC and metastatic properties. Altogether, our results suggest that MRR is critical in TNBC TIC character and stemness.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-08

Title: Identifying metastatic drivers in patient derived xenograft models of triple negative breast cancer

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Body: Metastases are responsible for the vast majority of deaths due to breast cancer. Triple negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterized by high rates of metastasis and poor response to chemotherapy. We are using patient derived xenograft (PDX) models of TNBC to identify drivers of TNBC metastasis. Using these models, we demonstrated that human breast tumors are capable of completing all stages of the metastatic cascade in mice, and metastatic lesions are observed in organs normally found in patients with metastatic breast cancer including lung, liver, bone, brain and lymph nodes. Lentiviral transduction was employed to express both bioluminescent and fluorescent proteins in three distinct PDX models of TNBC. In this way, metastatic lesions can be isolated using bioluminescent imaging and circulating tumor cells (CTCs) are isolated by flow cytometry. A lung metastasis gene expression signature was generated and comprehensive gain-of-function screens are being conducted *in vivo* to validate this signature and identify functional drivers of TNBC metastasis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-09

Title: MiRNA-200b suppresses triple negative breast cancer metastasis by targeting ARHGAP18 and causing sustained Rho A activation

Wang Z, Humphries B, Li Y and Yang C. Michigan State University, East Lansing, MI.

Body: Triple negative breast cancer (TNBC) refers to a group of highly heterogeneous malignant tumors that lack estrogen receptor and progesterone receptor expression, and human epidermal growth factor receptor 2 amplification, accounting for 10-20% of newly diagnosed breast cancer cases. Compared to other subtypes of breast cancer, TNBCs usually show worse clinical features such as rapid tumor growth, earlier recurrence, and more aggressive metastasis. Unfortunately, the mechanism of TNBC metastatic behavior has not been well understood. Moreover, no efficient targeted therapies for TNBCs are currently available, representing a real unmet need for effective new therapies. MicroRNAs (miRNAs) are a large family of small non-coding RNAs that negatively regulate protein-coding gene expression post-transcriptionally by interacting with messenger RNAs (mRNAs), causing mRNA degradation or translation inhibition. MiR-200 family members are among the first miRNAs reported to function as potent inhibitors of epithelial to mesenchymal transition (EMT), an important event in cancer metastasis. However, the effect of miR-200 family on TNBC metastasis has not been well understood. In this study, we investigated the effect of miR-200b, one member of the miR-200 family, on TNBC metastasis using cell culture and mouse orthotopic mammary xenograft tumor models. We found that the expression level of miR-200b is significantly lower in TNBC cells and tissues than that in other types of breast cancer. Stably expressing miR-200b significantly reduced TNBC cell migration and invasion and suppressed TNBC metastasis in a mouse orthotopic mammary xenograft tumor model. Mechanistic studies revealed that miR-200b overexpression in TNBC cells caused drastic changes in cellular actin cytoskeleton organization patterns as evidenced by reduced lamellipodia formation but increased stress fiber formation. In consistent with these findings, Rho GTPase pulldown assays demonstrated that stably expressing miR-200b significantly increased the Rho GTPase Rho A activation, but reduced the Rho GTPase Rac1 activation. Moreover, inhibition of Rho A signaling impaired the inhibitory effect of miR-200b on TNBC cell migration. Bioinformatics analysis indicated that ARHGAP18, a specific Rho A GTPase activating protein (GAP), is a predicate target of miR-200b. Further Q-PCR, Western blot and 3'UTR reporter analysis confirmed that ARHGAP18 is a target of miR-200b. Knocking down ARHGAP18 in TNBC cells using siRNAs significantly increased Rho A activation but reduced Rac1 activation. To further determine the role of ARHGAP18 in TNBC, ARHGAP knockout TNBC cells were generated using the CRISPR technology. It was found that knockout ARHGAP18 phenocopied the effect of miR-200b overexpression. Moreover, overexpressing ARHGAP18 in miR-200b stable expression cells overcome the inhibitory effect of miR-200b on TNBC metastasis. Together, these findings suggest that miR-200b suppresses triple negative breast cancer metastasis by targeting ARHGAP18 and causing sustained Rho A activation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-10

Title: Estrogen receptor $\beta 2$ and $\beta 5$ increase expression of ABCG2 and drug resistance of the triple- negative breast cancer cell line SUM159

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Body: Recent clinical studies indicate that estrogen receptor $\beta 2$ (ER $\beta 2$) and $\beta 5$ (ER $\beta 5$) expression in TNBC correlates to worse prognosis. We have expressed ER $\beta 2$ and ER $\beta 5$ in the triple negative cell line SUM159. Estrogen receptors $\beta 2$ and $\beta 5$ are highly similar to estrogen receptor $\beta 1$, except for a truncated C-terminus making the remaining ligand -binding domain incapable of binding to estrogen. In addition, at the C-terminus, ER $\beta 2$ and $\beta 5$ have a unique peptide each of 27 and 4 amino acids in length respectively. Stably expressing ER $\beta 2$ and ER $\beta 5$ using a transposon integrated, tetracycline- regulated expression system increases expression of the drug resistance gene, ABCG2 in the triple negative SUM159 cells. Furthermore, we also find that the cells become more resistant to treatments with paclitaxel. We find that ER $\beta 2$ and ER $\beta 5$ interact with HIF-1 α and HIF-2 α and are recruited to HIF response elements (HRE's) in chromatin. In agreement with this, we find that ER $\beta 2$ and ER $\beta 5$ are recruited to the ABCG2 promoter, which contains HRE's and is known to be a target of HIF signaling. We propose that ER $\beta 2$ and ER $\beta 5$ play a role in chemo - resistance of TNBC and that targeting these factors may reduce chemo resistance thus preventing tumor-relapses and their associated mortalities.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-11

Title: Distinct recruitment of tumor-associated immune cells correlates with increased pro-malignant chemokines in tumors expressing epithelial atypical chemokine receptor 1 (ACKR1/DARC), indicating a unique tumor microenvironment

Jenkins BD D, Martini RN N, Hire R, Monteil MA A and Davis MB B. University of Georgia, Athens, GA and AU/UGA Medical Partnership, Athens, GA.

Body: The breast tumor microenvironment is a complex assortment of cancer and host immune cells that interact to produce a myriad of clinical outcomes for breast cancer (BrCa) patients. Chemokine receptors, such as the *Atypical Chemokine Receptor 1 (ACKR1)*, play an important role in maintaining the homeostasis of pro-inflammatory chemokines, as well as influencing the migration of host immune cells. ACKR1 has the ability to modify the breast tumor microenvironment in multiple ways, including sequestering chemokine activity and affecting leukocyte migration. The purpose of this study is to characterize the variation in immune cell signaling between ACKR1 positive and negative tumors, and determine any correlation between ACKR1 and CCL2 (MCP-1) and CXCL8 (IL-8) in various BrCa cell types.

Primary breast tumor samples were stained using immunohistochemistry for ACKR1, in addition to various immune cells including, T-cells, B-cells, dendritic cells, and macrophages. We determined levels of circulating chemokines using a Luminex assay kit on whole blood lysate. We also tested the localization of ACKR1-associated chemokines and the abundance of ACKR1 using immunofluorescence techniques. Pilot data collected from primary breast tumor tissue suggested that differential expression of ACKR1 from various tumor subtypes leads to the recruitment of a specific subset of host immune cells to the tumor microenvironment. In our cohort, ACKR1 positive tumors tended to recruit B cells and dendritic cells to the site of the tumor, whereas ACKR1 negative cells did not. We also detected a positive correlation between ACKR1 levels in tumor tissue with CCL2 and CXCL8 levels in the circulating blood of our study group. Finally, co-localization of ACKR1 with CCL2 and CXCL8 was observed in cultured mammalian breast cancer cells. Overall, our pilot data suggests that there is differential recruitment of immune cells within the ACKR1 positive and negative BrCa tumor microenvironments, and that circulating CCL2 and CXCL8 concentrations are positively correlated with ACKR1 levels in BrCa cells.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-12

Title: Inhibiting ADP-ribosylation factor 1 activation to suppress breast cancer metastasis

Teng Y, Chavanieu A and Xie X. Augusta University; Georgia Cancer Center, Augusta University; Institut des Biomolécules Max Mousseron (IBMM) and Emory Children's Center, Emory University.

Body: ADP-ribosylation factor 1 (ARF1) is a crucial regulator in vesicle-mediated membrane trafficking and involved in the activation of signaling molecules. However, virtually nothing is known about its function in cancer metastasis. We show for the first time that *ARF1* is the most amplified gene in *ARF* gene family in breast cancer. Amplification of *ARF1* is associated with increased mRNA expression, and knockdown of *ARF1* leads to suppression of migration and invasion in breast cancer cells. The orthotopic xenograft model in NSG mice shows knockdown of *ARF1* in breast cancer cells inhibits pulmonary metastasis. The zebrafish-metastasis model confirms that the loss of *ARF1* expression suppresses breast cancer cells to metastatic disseminate throughout fish body. ARF1 function largely depends on its activation and there are no drugs that directly target *ARF1* gene expression. LM11, a cell-active inhibitor that specifically inhibits ARF1 activation through targeting the ARF1-GDP/ARNO complex at the Golgi, significantly impairs metastatic capability of breast cancer cell in zebrafish. These observations indicate that LM11 has potent anti-cancer activities. Here, we demonstrate that ARF1 is a very compelling target to limit metastasis, and inactivation of it could be a potential therapeutic approach to inhibit the late stage of breast cancer progression. Therefore, our study has significant impact on the design and execution of effective therapy of patients with high risk or metastatic breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-13

Title: BCAP31 promotes EMT and metastasis upon nutrient deprivation through autophagy in breast cancer

Fu W, Sun H, Gao S, Li L and Jin W. Key Laboratory of Breast Cancer in Shanghai, Collaborative Innovation Center of Cancer Medicine, Fudan University Shanghai Cancer Center, Shanghai, China and Shanghai Medical College, Fudan University, Shanghai, China.

Body: Reprogramming of cellular metabolism has regained substantial research interest over recent years. To survive, metastasizing cancer cells exhibit metabolic flexibility to acquire necessary nutrients from a frequently nutrient-poor environment and utilize these nutrients to both maintain viability and build new biomass. In present study, we found that B-cell receptor-associated protein 31 (BCAP31) was induced by the unfolded protein response pathway under energy stress. Despite its cleavage product, p20Bap31, has been reported to contribute to apoptosis, function of the entire protomer of BCAP31 was still unclear in cancer. Here, we demonstrated that up-regulation of BCAP31 under stress promoted tumorigenesis and increased the expression of epithelial-mesenchymal transition markers in breast cancer cell lines. Furthermore, overexpression of BCAP31 promoted the metastatic and invasive ability of breast cancer cells both *in vitro* and *in vivo*. These effects were associated with the unique role of BCAP31 in autophagy initiation. Depletion of BCAP31 or blockage of the autophagy process eradicated these tumorigenic effects. Mechanically, up-regulation of BCAP31, as a putative chaperone/quality control factor, triggered autophagy by aggregating JNK1 and the Bcl-2/Beclin 1 complex in endoplasmic reticulum (ER), therefore in turn increased the phosphorylation of Bcl-2, leading to its dissociation from Beclin 1. Taken together, the results uncovered a novel adaptive mechanism coupling ER cargo genes with the potential of malignant cells to expand their metabolic repertoire, and provided a broad framework to understand further the prevention of tumor progression.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-14

Title: Dissection of the Abl interactor 1 signaling in metastatic breast cancer cells

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Body: Abl interactor 1 (Abi1) is a key component of WAVE regulatory complex (WRC) that regulates actin cytoskeleton reorganization, membrane receptor signaling, and intracellular trafficking. Recent in vitro and in vivo studies as well as the studies of human patient samples suggest that Abi1 may play an important role in breast cancer (BC) metastasis. To determine if Abi1 is deregulated in metastatic breast cancer cells, we compared the expression of Abi1 in a panel of human breast cancer cell lines exhibiting distinct metastatic properties. In comparison with low-metastatic ER⁺/PR⁺ BC lines MCF-7 and T47D and a non-transforming mammary epithelial cell line MCF-10A, two ER⁻ BC lines CN34 and MDA 231 and their sublines selected by high metastatic potential to bone (231Bo), brain (231Br and CN34Br), and lung (231LM2) show significantly reduced Abi1 protein levels, with greater reduction found in highly metastatic sublines. It is unlikely that the down-regulation of Abi1 expression in these metastatic BC cells occurs at mRNA level, because RT-PCR analysis shows no significant difference in Abi1 mRNA levels among the cells analyzed. Interestingly, despite a reduction in protein level, the tyrosine phosphorylation of Abi1 was increased in CN34, MDA231, and their metastatic sublines compared to low-metastatic BC cells. The tyrosine phosphorylation of Abi1 is stimulated by epidermal growth factor (EGF) in these cells and Abi1 is recruited to EGF receptor (EGFR) upon EGF stimulation. The deregulation of Abi1 in metastatic BC cells is mediated by Abl tyrosine kinases, as the inhibition of Abl family kinases by imatinib not only attenuates EGF-stimulated Abi1 tyrosine phosphorylation but also rescue the down-regulation of Abi1 protein in metastatic BC cells.

These findings are consistent with a role of the EGFR-Abl-Abi1 signaling in BC metastasis. The studies suggest that this pathway may serve as a biomarker for diagnosis and/or prognosis of metastatic breast cancer. We have identified the tyrosine 213 in Abi1 as a major phosphorylation site stimulated by Abl kinases and have generated a unique conditional Abi1 depletion/re-expression system to dissect how the deregulation of the Abi1 signaling contributes to BC metastasis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-15

Title: Novel regulation of breast cancer cell aggressiveness by cancer testis antigen

Kannan A, Wells RM M, Ikebe M and Dasgupta S. The University of Texas Health Science Center; The University of Texas Health Science Center and The University of Texas Health Science Center.

Body: We recently demonstrated that a vesicular endocytosis associated protein SH3GL2, attenuates spontaneous metastases of breast cancer cells by inducing a mesenchymal to epithelial differentiation and the onset of the intrinsic apoptotic pathway. The present study aims to understand the molecular mechanism behind the SH3GL2 mediated reduction of spontaneous metastasis of the breast cancer cells.

We employed a cDNA microarray analysis of the SH3GL2-overexpressing breast cancer cells exhibiting reduced pulmonary metastasis and identified a 12.1 fold downregulation of *SPANXB1*, a cancer-testis antigen that regulates sperm motility. A limited number of studies reported an association between increased SPANXB1 expression and progression of melanoma and hepatocellular carcinoma. Augmented SPANXB1 mRNA and protein expression was evident in primary breast tumors and its upregulation was associated with pulmonary metastasis of breast cancer cells. However, the expression pattern of SPANXB1 and its role in BCa development and progression is unknown. By immunohistochemical analysis, we detected high expression ($p=0.002$) of SPANXB1 in 78% (18/23) of the primary breast cancer tissues and corresponding lymph node metastases compared to the matched normal breast tissues. A couple of non-tumorigenic human breast epithelial cell lines were stably transformed with SPANXB1 to understand its effect on cellular growth and progression. The SPANXB1-transformed cells exhibited increased invasion ($p=0.0001$) and epithelial to mesenchymal transition accompanied by an augmented expression ratio of Vimentin/E-Cadherin, molecules regulating differentiation and metastasis. The SPANXB1-transformed cells also exhibited a markedly reduced expression of SH3GL2, implicating a SPANXB1:SH3GL2 crosstalk accompanied by an enhanced production of lactate ($p=0.004$). Our investigation identifies new breast cancer promoting role of a cancer testis antigen, which bears potential for biomarker and targeted therapeutic development.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-16

Title: The phosphorylation-specific association of STMN1 with GRP78 promotes breast cancer metastasis

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Body: Background

Stathmin 1 (STMN1) is a phosphoprotein associated with cancer metastasis. The activity of STMN1 can be modulated by its phosphorylation of multiple Serine residues (Ser16, Ser25, Ser38, and Ser63). In this study, we examined STMN1 expression and the phosphorylation status and explored the related signaling mechanism in breast cancer metastasis.

Methods

A phospho-specific protein microarray containing 1318 phosphoprotein antibodies was used between MDA-MB-231 and highly metastatic variant MDA-MB-231-HM cells. STMN1 and phospho-STMN1 signatures were assessed by immunohistochemistry of 310 patients and cell migration tests of different alanine-substituted STMN1 mutants. Tandem affinity purification followed by mass spectroscopy and kinase inhibitors treatment were used to explore the related signaling mechanism and finally confirmed in mouse models.

Results

High expression of STMN1 and phosphorylation of STMN1 at Ser25 and Ser38 are necessary to maintain cell migration capabilities and is associated with shorter disease-free survival (DFS) in breast cancer, while Ser16 and Ser63 phosphorylation showed the opposite effects ($P < 0.01$). Mass spectroscopy showed that glucose-regulated protein of molecular mass 78 (GRP78) bind to wild type and mutant S16A/S63A but not S25A/S38A STMN1. Conversely, mutations at Ser25 and Ser38 significantly reduced STMN1 binding to GRP78. Then we selected mitogen-activated protein kinase (MEK) kinase inhibitor PD0325901 significantly reduce both Ser25 and Ser38 phosphorylation and GRP78-STMN1 binding. Suppression of GRP78 expression reduced the migration of MDA-MB-231 cells expressing wild type but not S25A or S38A mutant STMN1 ($P < 0.01$). These findings suggested that MEK kinase phosphorylation at Ser25 and Ser38 is required for GRP78 binding to STMN1 and that GRP78 binding to phosphorylated STMN1 is necessary for cell migration. *In vivo* mouse models showed that 20% of the hosts in the S25A/S38A group developed metastasis compared to the wild type control and the S25A/S38A mutant group had lower GRP78 expression levels. A prognostic p-STMN1/GRP78 signature was also established by a Cox proportional hazards model: risk score = $-0.680 \times \text{Ser16} + 0.722 \times \text{Ser38} - 0.636 \times \text{Ser63} + 0.899 \times \text{GRP78}$. The prognostic accuracy of the risk score was assessed by using a time-dependent ROC analysis and found that it has a higher prognostic accuracy compared to the TNM staging system (area under the ROC curve (AUC) for the p-STMN1/GRP78 model: 0.792; AUC for TNM staging: 0.674; $P = 0.023$)

Conclusion

In this study, we investigated the metastasis-specific phosphorylation states of STMN1 and their correlation with clinical outcomes. GRP78 was identified as a novel phospho-STMN1 binding protein upon STMN1 Ser25/Ser38 phosphorylation and this phosphorylation-dependent interaction is regulated by MEK kinase. We also propose a prognostic model based on phospho-STMN1 and GRP78 to assess metastatic risk which could enable oncologists to target breast cancer patients for appropriate treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-17

Title: Epithelial paradox; clinical significance of co-expression of E-cadherin and vimentin in the invasion and the metastasis of breast cancer

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Body: Background: E-cadherin and vimentin are now regarded as major and conventional canonical markers of epithelial-mesenchymal transition (EMT). It is commonly assumed E-cadherin is uniformly lost during the process of EMT. We previously reported that the elevated expression of vimentin contributes to the aggressive phenotype in invasive breast cancer. On the other hand, the role of E-cadherin in breast cancer biology might be unclear and more complex. Although, cell cohesion during breast cancer invasion is often overlooked, accumulating evidences indicate breast tumor cells are typically cohesive and often display membrane-localized E-cadherin in both the primary tumor and distant metastases, termed collective invasion. Multiple mechanisms have emerged to address how epithelial breast tumors invade.

Aims: The aim of this study is to reveal the clinical importance of the expression pattern of E-cadherin and vimentin in breast cancer.

Methods: The E-cadherin and vimentin protein expression were evaluated by immunohistochemistry (IHC) in 177 invasive breast cancer samples. Among these, E-cadherin and vimentin expression were evaluated in the set of primary breast cancer and metastatic lymph nodes in 65 cases. In addition, E-cadherin and vimentin expression were analyzed by immunofluorescent staining and evaluated using confocal laser scanning microscopy to see E-cadherin and vimentin localization in the breast cancer cells.

Results: The positive vimentin expression was highly correlated with poor disease-free survival (DFS) and overall survival (OS) ($p=0.019$ and $p=0.0044$), however, the E-cadherin expression alone did not correlate with prognosis. Interestingly, both E-cadherin and vimentin positive tumors had the worst DFS and OS among all breast cancer ($p=0.03$ and $p=0.0089$). Vimentin expression was highly correlated between primary tumors and metastatic lymph nodes. However, E-cadherin expression levels were significantly elevated in metastatic lymph nodes ($p=0.0017$). Co-expression of E-cadherin and vimentin in the metastatic lymph nodes also showed worst DFS and OS ($p=0.12$ and $p=0.027$). Immunofluorescent analysis revealed that E-cadherin and vimentin were co-localized within the same tumor cells in many of the E-cadherin high/vimentin positive tumors.

Conclusions: Co-expression of E-cadherin and vimentin seems to be associated with the most aggressive phenotype and poorest prognosis in breast cancer. Moreover, co-localization of E-cadherin and vimentin within the same breast cancer cells suggests the significance of the expressions of both proteins in breast cancer invasion and metastasis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-18

Title: Early detection of development of a pre-metastatic niche in lungs in response to primary breast tumor using Raman spectroscopy

Zheng C, Rizwan A, Paidi SK Kumar, Yu Z, Barman I and Glunde K. The Second Hospital of Shandong University, Jinan, Shandong, China; The Johns Hopkins University In Vivo Cellular and Molecular Imaging Center, The Johns Hopkins University School of Medicine, Baltimore, MD and Johns Hopkins University, Baltimore, MD.

Body: Background: An alarmingly large proportion of cancer-related deaths result from metastatic cancers. Development of quick, reliable and non-invasive or minimally invasive approaches to objectively assess the secondary tissues (potentially in vivo) will be instrumental in substantially reducing the cancer burden due to metastasis, which accounts for majority of cancer related mortality. In our study we report the utilization of Raman spectroscopy and chemometric techniques in identifying formation of pre-metastatic niche in lungs prior to observing morphological changes.

Methods: Six-week-old female athymic nu/nu mice (NCI, MD) were implanted with 2×10^6 cells of human breast cancer cell lines - MDA-MB-231 (n=3), and MCF-7 (n=3) in their fourth right mammary fat pad orthotopically. And control mice (n=3) without tumor cell implantation were also employed. The primary tumor size was monitored and the mice were sacrificed within 8-12 weeks of cell implantation when the primary tumors volume grew to 500-600 mm³. Control mice were also sacrificed in this timeframe. The freshly excised lungs of the mice were cleaned in PBS and utilized for obtaining Raman spectra (830 nm, thermoelectrically cooled CCD). Each tissue was collected from multiple points. Principal component analysis (PCA) and Partial least squares discriminant analysis (PLS-DA) were employed as discriminating algorithm. Following spectral acquisition, the tissues were fixed in 10% formalin, embedded in paraffin, and then HE staining, and Masson's trichrome staining for collagen. Collagen quantification of Masson's trichrome stained slides was achieved using MATLAB (Mathworks, MA). The Institutional Animal Care and Use Committee at Johns Hopkins University School of Medicine approved the protocol of the study.

Results: 900 Raman spectra each acquired from the lungs of the control mice and mice bearing MCF-7 and MDA-MB-231 tumor xenografts were assigned class labels - 'Control', 'MCL' and 'MDL' respectively for further analysis. Select principal components from those obtained by subjecting all the chosen 900 spectra to PCA clearly evident that the differences in the Raman spectra belonging to tissues being primed by derivatives of different primary cells are quite pronounced. The average correct rates of PLS-DA prediction of 90.1%, 97.7% and 78.4% were obtained for the spectra belonging to the classes - Control, MCL and MDL respectively. The HE images are negative for any signs of cancerous lesions. Masson's trichrome staining results show that the metastatic potential of the cell lines responsible for the primary tumor is positively correlated with the collagen density in the pre-metastatic niche, the MDL shows the highest collagen density (P<0.001). These differences clearly indicate the remodeling of extracellular matrix anticipating incoming tumor cells in response to primary tumor derived factors occurs very early in the metastatic cascade.

Conclusion: The current study introduces Raman spectroscopy in conjunction with chemometric techniques as are liable and minimally invasive tool for diagnosis of metastatic cancers significantly early in the metastatic cascade, and also opens a new route for early targeting of cancer metastasis and its associated burden.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-19

Title: Decoding the genetic basis of mammary mineralization and their putative role in promotion of distant metastases

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Body: Background: Breast microcalcifications are the sole early stage diagnostic markers of breast cancer. The association of mineralization (especially type II microcalcifications) with both benign and malignant lesions often leads to unnecessary biopsies. The processes by which these ectopic microcalcifications form are unknown. In the current work, we attempted to explore the possibility of obtaining genes responsible for the formation of microcalcifications in breast cancer cell lines at cellular level and understand their potential involvement in disease progression and distant metastases.

Methods: The GEO dataset GSE16795 used in this study contains gene expression profiles of 28 human breast cancer cell lines that were divided into two groups - metastatic and non-metastatic. Gene expression levels of OPN were found to be significantly ($p=0.0002$) elevated for the metastatic group compared to the non-metastatic group. Hence, the human breast cancer cell lines - metastatic (MDA-MB-231 and SUM 149) and non-metastatic (BT-474 and T47D) from the American Type Culture Collection were cultured and their OPN expression at mRNA and protein levels determined by qRT-PCR and Western blotting were compared. Additionally the same cell lines were cultured in media enriched with an osteogenic cocktail containing 10mM β -glycerophosphate (Sigma, USA) and 50 mg/ml⁻¹ ascorbic acid (Sigma, USA) for induction of microcalcifications. Next, several clones were generated using shRNA knockdown of OPN gene in MDA-MB-231 cells for further study. *In vitro* studies were conducted to assess the effects of OPN knockdown on the migration and invasion potential of MDA-MB-231 cells using transwell migration assays.

Results: The expression of OPN at both mRNA and protein levels are significantly higher for the metastatic cell lines when compared to non-metastatic cell lines. It can also be observed that OPN expression in the cells increases substantially with addition of exogenous phosphates in the form of osteogenic cocktail and thereby indicating that OPN possibly plays a crucial role in mediating formation of microcalcifications in these cells ($P<0.001$). Metastatic cell line MDA-MB-231 was employed as a model system for further investigation in this study. There is a consistent inhibition of formation of cellular microcalcifications due to the knockdown of gene responsible for OPN, suggesting that OPN gene is directly associated with the formation and regulation of hydroxyapatite formation in MDA-MB-231 breast cancer. Further, the above observation is strengthened by the similarity of the trend of variation in level of mRNA expression of OPN and cellular calcification content across the knockdown clones. OPN knockdown cell lines show reduced cell migration and invasion in the assays compared to the control MDA-MB-231 cells.

Conclusion: The knockdown of OPN gene not only reduced the formation of microcalcifications in the cells in response to osteogenic cocktail but also affected their migration and invasion characteristics. The observed dual roles of the OPN gene encourage us to probe further into the possible existence of a direct relationship between microcalcifications and ability to metastasize to distant organs mediated by common genetic factors in the future.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-20

Title: Effects of local and radiation enhanced TGF β on the invasive nature of inflammatory breast cancer cells

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Body: Inflammatory Breast Cancer (IBC) is the deadliest form of epithelial breast cancer, accounting for ~10% of breast cancer deaths in the United States. A hallmark of IBC is the formation intralymphatic emboli that are known to be chemotherapy and radiation resistant and contribute to rapid metastasis. This form of breast cancer progresses very quickly, expressing aggressive behavior. IBC and melanoma share a number of similarities in disease presentation and progression. Both spread via dermal lymphatics, form intralymphatic emboli and have a propensity to form cutaneous metastases. Melanoma can also present as “inflammatory melanoma”, which resembles IBC phenotypically. Thus, new leads for studying cutaneous metastasis can be gathered from the melanoma literature. Studies demonstrate a role for transforming growth factor beta (TGF β) in the etiology of melanoma cutaneous metastasis. TGF β promotes tumor cell invasion and its expression can be induced in the stroma by radiation treatment. Recently, our lab has demonstrated low expression of TGF β in IBC patients, which we believe promotes cohesive invasion of IBC cells. Stimulation of IBC cells with 2ng/ml of TGF β causes altered tumor cell behavior such as stimulating single cell invasion. The invasion of KPL4, SUM149 and MDA-MB231 cell lines were significantly higher in TGF β stimulated cells compared to non-stimulated cells. As in melanoma cells we hypothesize that radiation enhanced local TGF β production in the stroma. We radiated normal human epidermal fibroblasts cells with 0gy, 0.5gy, 1.0gy, 2.5gy and 5.0gy intensity and observed that the invasion was significantly higher in 1.0gy, 2.5gy and 5.0gy. We have also looked at the levels of TGF β -1 in different conditioned medium. ELISA results don't show significant level of increase in TGF β -1. Since there are two more TGF β receptors in the TGF β pathway (TGF β -2 TGF β -3), next I will look at the level of TGF β -2 and 3 on the different intensity of radiation-conditioned medium. Our prediction is that the increase in the invasion of IBC cells is because of TGF β , which alters the cohesive nature of IBC cells, and enhance single cell invasion. Moreover, radiation increases TGF β levels in the stroma, which is responsible for rapid metastasis of IBC cells to the skin.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-01-21

Title: Novel CD146-downstream signaling pathway involved in breast tumor suppression

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Body: While CD146 is a promoter of various tumor types, its true function in breast cancer (BC) is still controversial. However, evidence from our work and others indicate that CD146 acts as a tumor suppressor in BC. To test this hypothesis, we developed both *in vitro* and *in vivo* tetracycline (tet On)-inducible system of CD146 using MDA-MB-231 founder BC cell line. Our results demonstrated that induction of CD146 suppressed BC cell migration and invasion *in vitro* as well as tumor growth and progression in mouse breast xenograft model. Microarray gene expression profiling revealed latexin (LXN: a variant of Tissue Inhibitor of Metalloproteinases) as a novel potential CD146-downstream signaling transcriptional target, which was validated using various *in vitro* approaches. To further validate our finding, immunohistochemical analysis of breast tumor tissues from both human and mouse (tet-inducible system) breast tissues showed that, while the expression of both CD146 and LXN were highly expressed in the early stages of BC (normal and benign tissues), it was lost in advanced stages (malignant and metastatic tissues). Pharmacological approach combined with luciferase assay revealed that NF κ B activation *via* Akt pathway couples CD146 to the transcription of LXN in BC CD146-inducible cells. The present study discovers the main molecular players of a novel signaling pathway that underpin CD146-suppressed BC progression.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-02-01

Title: Metabolic stress induces GD2 expression and cancer stem cell phenotype in triple negative breast cancer

Battula VL, Piyarantna B, Nguyen K, Sun JC C, Jin F, Coarfa C, Nagireddy P and Andreeff M. The University of Texas MD Anderson Cancer Center, Houston, TX and Baylor College of Medicine, Houston, TX.

Body: Breast cancer stem cells (BCSCs) have been characterized as a fraction of cells in primary tumors that are drug resistant and have metastatic potential. Ganglioside GD2 has been shown by us and others as a marker for BCSCs. Furthermore, nutrient deprivation associated metabolic stress seen during tumor progression is reportedly associated with the cancer stem cell phenotype. We hypothesized that metabolic stress could induce spontaneous generation of GD2⁺ BCSCs during tumor progression. To test our hypothesis, we cultured breast cancer cell lines MDA-MB-231 and SUM159 at low seeding density and measured percentage and absolute number of GD2⁺ cells daily. Flow cytometry analysis revealed that the percentage of GD2⁺ cells increased from 4.5 ± 2.5 on day 2 to $15 \pm 3.8\%$ on day 5 in MDA MB-231 cells and from $8.5 \pm 2.8\%$ on day 2 to $28 \pm 6.2\%$ on day 5 in SUM159 cells (both designated as triple-negative breast cancer, TNBC). To investigate this phenomenon in-vivo, we injected GFP⁺ MDA-MB-231 cells in NSG mice mammary fat pads and examined GD2 expression in the implanted tumors weekly. Interestingly, we noticed that the percentage of GD2⁺ also increased from $12 \pm 1.5\%$ on week 1 to $30 \pm 2.5\%$ on week 6. Next, SUM159 cells were cultured in either nutrient rich (NR, i.e., 10% serum) or nutrient deprived (ND, 1% serum) for 4 days. We found that the percentage of GD2⁺ cells in NR medium at the end of 4 day culture was ~20% of the total cell population, whereas in ND medium was almost 50%. We then tested the effects of nutrient rich environment on GD2 expression by refreshing the media daily. Interestingly, cells that received fresh media had lower number of GD2⁺ cells ($15 \pm 1.5\%$) compared to cells cultured in the same medium for 4 days ($33 \pm 2.5\%$). Our data suggests that nutrient deprivation induces a stem cell phenotype in TNBC cells.

Next, we performed global metabolic profiling (i.e., for a total of 300 biochemical metabolites) using a mass spectroscopy-based approach. We profiled SUM159 cells cultured with NR vs. ND medium (set-1); GD2⁺ vs GD2⁻ SUM159 cells (set -2); GD2⁺ vs GD2⁻ MDA-MB-231 cell (set-3). Metabolites associated with amino acid metabolism, in particular glutathione metabolism, including glutamyl-alanine, 5-oxy-proline, proline, glutamine, and glutathione itself were found to be most highly up-regulated in GD2⁺ compared to GD2⁻ cells and also in cells cultured in serum starved compared to serum rich conditions. Further analysis of these metabolites and their association with GD2⁺ cell signature revealed that gamma-glutamyl transferase (GGT5), was one of the most highly up-regulated (>150-fold) gene across all the groups. GGT is expressed on cell surface and transfers glutamyl group to amino acids, which then get transported across the membrane. In cancer, cells expressing GGT has been shown to be resistant to chemotherapeutic agents including cisplatin. Targeting glutathione metabolism could be future therapeutic strategy to inhibit BCSC growth in TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-02-02

Title: Uridine diphosphate glucose dehydrogenase is required for hyaluronic acid production and breast cancer invasion

Arnold JM M, Rasaily U, Ramirez-Peña E, Pathak R, San Martin R, Purwaha P, Rao A, Putluri N, Rowley D, Sikora A, Mani S and Sreekumar A. Baylor College of Medicine, Houston, TX; MD Anderson Cancer Center, Houston, TX and BSW Memorial Hospital, Temple, TX.

Body: Breast cancer is a significant public health concern and there remain unmet challenges in the diagnosis and treatment of triple negative breast cancer (TNBC). Gene expression profiling has revealed that TNBC is composed of a diverse set of disease states and thus there is a need for a more integrated approach to describe phenotypic subtypes within TNBC. Metabolism, as the integrated product of upstream signaling events, environmental conditions and energetic demands, is deeply linked to cellular phenotype. In recent years it has been unequivocally demonstrated that cancers exhibit altered metabolism compared to normal tissues, and furthermore many of these alterations are potentially actionable therapeutic targets. In a recent publication we described several metabolic alterations which occur during the epithelial-mesenchymal transition (EMT) and demonstrated these alterations could be used to define a prognostic metabolic signature in breast cancer patients. Following up on that work, I identified a set of metabolic enzymes which are specifically upregulated during EMT and are significantly upregulated in a subset of TNBC patients with mesenchymal-like disease. Using genetic and biochemical approaches, I have identified one of these enzymes, uridine diphosphate glucose dehydrogenase (UGDH), to be necessary for EMT and cellular invasion. UGDH facilitates the rate-limiting step in the production of cellular uridine diphosphate glucuronic acid (UDP-GlcUA), a precursor for hyaluronic acid (HA) and other glycosylaminoglycans. HA production has previously been identified as an important process for EMT and cancer progression. Here we demonstrate that RNAi-mediated depletion of UGDH significantly decreases HA production and inhibits breast cancer invasion in both *in vitro* and *in vivo* models. Additionally, we demonstrate the compound 4-methylumbelliferone (4-MU), which inhibits HA production via depletion of intracellular UDP-GlcUA, significantly inhibits cellular invasion, colony formation, and partially reverses EMT. Taken together, this research indicates that UGDH and intracellular UDP-GlcUA may represent novel, pharmacologically actionable therapeutic targets for the inhibition of breast cancer invasion.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-02-03

Title: Leptin receptor (OB-R) in breast carcinoma tissue: Ubiquitous expression and correlation with leptin-mediated signaling, but not with systemic markers of obesity

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Body: Background/Aims: Obesity is associated with a 30-50% increased risk of breast-cancer (BC) mortality, most consistently in estrogen receptor (ER) positive disease, through unclear mechanisms. Leptin is a multi-functional protein with key actions on adipose tissue. In pre-clinical studies, leptin stimulates the growth, survival, and progression of BC cells through both estrogen dependent and other (e.g. JAK/STAT, PI3K/Akt, MAPK) pathways. Leptin has also been associated with increased BC risk and poor prognosis. Our aim was to correlate tumor leptin-receptor (OB-R) expression with tissue markers of cell signaling and systemic markers of obesity, inflammation, and metabolism in a cohort of ER+/HER2- BC patients.

Methods: From our biorepository, we identified ER+/HER2- BC patients having both blood and tissue samples available. Data included BMI, menopausal status, and family/cancer/medical history, tumor histology, grade, stage, and ER/PgR/HER2 status. We performed blood assays for factors related to inflammation, tumor growth, hormonal regulation, and metabolism (see below). Immunohistochemistry for OB-R, pAkt (S473), pERK (T202/Y204), and insulin-receptor (IR) was performed on archived tissue, and scored for % positive cells and intensity of staining. Allred and H-scores were calculated. Associations with OB-R scores were calculated using Pearson, Spearman, and χ^2 methods.

Results: 129 patients were eligible; 69.8% were post-menopausal and mean BMI was 27.8 ± 6.5 kg/m². Most tumors were no-special-type (79%), PgR+ (90%), and node-neg (78%). The tissue expression of OB-R and other markers was scorable in 118 (91%) cases.

OB-R was expressed in all 118/118 cancers (Allred score range: 3 to 8; median 7, mean 6.61). High blood leptin did not downregulate OB-R (Spearman $R=0$), even though leptin was strongly correlated with BMI (Pearson $r=0.78$, $p<0.00001$). Increasing OB-R correlated with phosphorylation of Akt ($R=0.19$) but not ERK ($R=0.08$). By contrast, high BMI was associated with lower Akt ($R=-0.18$) and ERK ($R=-0.11$) phosphorylation.

OB-R correlated with ER (Spearman $R = 0.27$), PgR ($R=0.29$), and insulin receptor ($R = 0.24$), weakly correlated with estradiol (Spearman, $R=0.11$) and fasting glucose ($R=0.18$), and negatively correlated with systemic IL-2 ($R=-0.11$) and IL-6 ($R=-0.21$). OB-R was not correlated with other blood markers (insulin, HOMA, PAI-1, IL-1ẞ, IL-8, VEGF, EGF, TNF- α , hsCRP, SHBG, or estrogens) or tumor grade.

Conclusions: OB-R is highly expressed in breast tumor tissue even in non-obese patients. Although leptin and BMI did not modulate OB-R expression, downstream signaling (e.g. Akt, ERK) did show a BMI-dependent effect, albeit of limited magnitude. This suggests that leptin acts on breast cancer cells through OB-R activation and downstream Akt/ERK signaling, without a coupled change in total OB-R expression. Further work is needed to elucidate the roles of inflammation, estrogens, and regulatory mechanisms within the PI3K-PTEN and Ras-MAPK cell-signaling networks.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P6-02-04

Title: Atorvastatin insensitivity is associated with increased lipid droplets accumulation and fatty acid metabolism in breast cancer cells

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Body: Lipophilic statins, including atorvastatin, may exert significant antiproliferative and proapoptotic effects in breast cancer as demonstrated in both clinical trials and cell models. However, heterogeneity in treatment response still remains a noteworthy challenge to be addressed. In this regard, we previously proposed a multigene signature including genes involved in cholesterol biosynthesis, which were dysregulated upon atorvastatin treatment and was shown to predict statin responsiveness in breast cancer (BC) cell lines and primary tumors. To further delineate the molecular mechanisms underlying this variability, we sought to characterize the differential statin-induced effects on intracellular lipid regulation observed in BC cell lines based on their sensitivity to atorvastatin treatment. BC cells were classified as insensitive (MCF-7 and T47D) or sensitive (MDA-MB-231) to atorvastatin by virtue of growth inhibition rate consequent to treatment with doses ranging up to 10 μ M. Under complete culture conditions, atorvastatin-induced decrease in cell proliferation was inversely correlated to a progressive accumulation of neutral lipids in lipid droplets (LDs) in the insensitive cells following 72 hours treatment. Interestingly, in the sensitive MDA-MB-231 cells no significant change in LDs formation was observed despite the very potent antiproliferative effects (60% impairment of growth). However, in correspondence of severe inhibition of growth rate of at least 80% by atorvastatin treatment, MDA-MB-231 cells displayed a consistent reduction in LDs accumulation, suggesting beneficial effects of LDs biosynthesis on proliferation of BC cells. Transcriptional profiling of genes involved in lipid metabolism using microarrays and validated by qRT-PCR ruled out the likelihood that atorvastatin treatment altered lipid uptake and export mechanisms in sensitive cells in the presence of exogenous lipid supply. Nevertheless, gene ontology analysis indicated that an induction of cell stress responses was likely associated to atorvastatin sensitivity. Significant deregulation of genes involved in the fatty acid metabolic process, including biosynthesis of monounsaturated fatty acids (MUFAs), and cholesterol biosynthesis were instead linked to atorvastatin insensitivity. Accordingly, we found that the magnitude of the induction of the mRNAs of stearoyl-CoA desaturase (SCD), the key effector in MUFAs metabolism, and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), the established target of statins, were consistently lower in sensitive cells compared to the insensitive counterpart in response to atorvastatin treatment. Therefore, we suggest that the ability to significantly increase the number of stored neutral lipids in response to statin treatment may likely confer a proliferative advantage to BC cells. Our results also identified MUFAs metabolism as an attractive pathway to be further investigated in view of finding promising biomarkers for unraveling the molecular basis of statin sensitivity in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-02-05

Title: Modulation of FASN under obese conditions

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Body: Introduction: Obesity is known to be associated with a worse breast cancer prognosis, in part through altering metabolism in cells of the tumor microenvironment. In particular, changes in metabolism associated with fatty acid utilization have been noted in not only breast cancer, but also several other cancer types. This includes changes to both expression and activity of the Fatty Acid Synthase enzyme (FASN), which is responsible for production of long chain fatty acids, including palmitate. These changes in long chain fatty acid production can modulate tumor behavior through modulation of energy utilization such as beta-oxidation, as well as plasma membrane modulation with phospholipids. Our previous studies have demonstrated that exposure to obese conditions induces significant changes in breast cancer cell proliferation. Additionally, obesity modulates activity of other cells within the tumor microenvironment, including adipocytes, which might influence the cancer cell itself. We hypothesize one particular mechanism that supports these changes is obesity-induced upregulation of FASN and that FASN may be a viable target to limit obesity-induced progression.

Methods and Results: FASN has been shown to promote cancer cell proliferation through generating fatty acid precursors required for cell proliferation, altering membrane fluidity, and activating oncogenic signaling pathways. To determine if modulation of FASN is an important mechanism by which obesity promotes disease progression, MCF-7 breast cancer cells and human pre-adipocyte cells (ASC) were exposed to 2% sera from obese postmenopausal women and 2% sera from non-obese (control) women. Preliminary quantitative PCR results demonstrated that exposure to the obese sera resulted in increased expression of FASN in both the cancer cells as well as the ASC. Current studies are on-going to determine if 1) FASN up-regulation results in increased long-chain and free fatty acid production in both the cancer and adipocyte cells, 2) whether changes in long chain and free fatty acid production results in altered metabolism and plasma membrane status and 3) whether targeting FASN with a new generation of FASN inhibitors currently being investigated in the clinic can modulate obesity-induced disease progression.

Conclusions: Our findings indicate that obesity promotes upregulation of FASN in several cells within the tumor microenvironment, including adipocytes and the cancer cell itself. Our on-going studies will confirm if this is an important mechanism by which obesity promotes disease progression. Since FASN inhibitors are currently being investigated in the clinic, the results of these studies will provide a better understanding of how obesity alters the biology of the disease, and may identify a novel target for improving patient outcomes.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-02-06

Title: Metabolomic profiling of breast cancer and adjacent tissue

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Body: Breast cancer (BC) is the most common cause of death among women worldwide. Nowadays, research directed toward the discovery of cancer molecular characteristics has extended into various biological aspects, from early investigations of cancer genomics and proteomics to recent efforts in cancer metabolomics. We consider that breast cancer cells display significantly altered cellular processes, and thus metabolites, compared to normal cells. In this regard, the application of metabolomics towards cancer research can lead to the discovery of metabolite cancer biomarkers and the identification of target therapeutics. **Methods:** Metabolomics signature was extracted from primary BC and adjacent tissue samples from a cohort of 182 breast cancer patients from the Institut Gustave-Roussy and Institute Curie – Paris, University of Debrecen – Hungary, and University of Tartu – Estonia. The Biocrates Life Science® company, performed the metabolomics analysis using the Absolute IDQ p180 kit. Statistical analysis was performed by MetaboAnalyst 3.0 software ($p < 0.05$). **Results:** We observed 99 differential metabolites between breast tumor and adjacent tissues ($p < 0.05$). In the tumor tissue we observed an increase of glycolysis, glutamine uptake and synthesis, glutamate production, intermediates for redox homeostasis, as well reduction in tricarboxylic acid cycle and β –oxidation impairment. Together, these pathways, favor lipogenesis with a consequent increase in saturated fatty acids and cholesterol synthesis for the formation of membranes and lipid rafts in tumors. The increase in rafts in tumors maintains proliferation signaling via membrane receptors, making them important biomarkers. **Conclusion:** Metabolomics analysis is useful in identifying differential metabolites between breast cancer and adjacent tissue. In this scenario, cancer cells may be dependent on some metabolites for its development, making them therapeutically valuable biomarkers.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-02-07

Title: Metabolomic analysis by nuclear magnetic resonance spectroscopy discriminates hormone receptor positive/HER2 negative breast cancer cell lines resistant to palbociclib

Bonechi M, Guarducci C, Meoni G, Tenori L, Biagioni C, Schiff R, Osborne CK Kent, Luchinat C, Di Leo A, Malorni L and Migliaccio I. Hospital of Prato, Azienda USL Toscana Centro, Prato, Italy; CIRMMP, University of Florence, Italy; Hospital of Prato, Azienda USL Toscana Centro, Prato, Italy; Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX and University of Florence, Italy.

Body: Introduction: Clinical trials of palbociclib in combination with endocrine therapy have recently shown unprecedented activity for the treatment of hormone receptor positive/HER2 negative (HR+/HER2neg) advanced breast cancer. However, de novo and acquired resistance to palbociclib limit its clinical utility. Moreover, combining palbociclib with endocrine therapy increases toxicity and costs of the treatment. Identifying patients more likely to benefit from this compound and understanding the mechanisms of resistance to palbociclib is critical. In this study we investigated whether metabolomic profiles of breast cancer cell lines with acquired resistance to palbociclib (PDR) differ from their sensitive counterpart (PDS). In addition we sought to identify metabolic biomarkers of sensitivity to palbociclib by analyzing a breast cancer cell line unable to acquire resistance to palbociclib.

Material and methods: We have established in our lab three PDR HR+/HER2neg breast cancer models (MCF7L, T47D and ZR75-1) by chronically exposing cells to escalating doses of palbociclib. PDR derivatives show IC50 values 6 to 30 times higher than their PDS counterparts. One additional model, CAMA-1, was unable to develop resistance. Whole-cell lysates and conditioned cell culture media from five replicates of each of the PDS and PDR models and from CAMA-1 were analyzed by nuclear magnetic resonance (NMR). Principal component analysis (PCA) was used as first exploratory analysis and as dimension reduction technique. Canonical Analysis (CA) was used to discriminate different groups. Differentially expressed metabolites between PDR and PDS models and between CAMA-1 and PDS cells were analyzed.

Results: Unsupervised PCA analyses of ¹H NMR spectra, in which no information about PDS and PDR was inserted in the statistical model, correctly identified individual cell lines on both whole-cell lysates and conditioned media. However this analysis did not discriminate PDS from PDR within each model. Using a supervised approach, in which the statistical model was trained to discriminate between PDS and PDR, these groups were categorized with accuracy of 80% using whole-cell lysates and of 65% using conditioned media, using a cross-validation analysis by repeatedly testing the model on blind samples. CAMA-1 was correctly identified as a PDS model; however it showed a distinct metabolic profile compared to other PDS models. Over 30 metabolites were identified as differentially expressed between PDS and PDR models in lysates and conditioned media, but only glycerophosphocholine levels in conditioned media remained significantly higher in PDR compared to PDS models after correction for multiple testing.

Conclusions: In this study we show that analysis of metabolic profile of cells lysates discriminates PDR from PDS cell lines with a high accuracy. Analysis of metabolic pathways implicated in resistance/sensitivity to palbociclib is ongoing and might help identifying new targets to overcome resistance. Additionally, metabolites associated with palbociclib resistance may be potentially tested in clinical samples as biomarkers for patients stratification. Further studies are warranted.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-02-08

Title: Modulation of indoleamine 2, 3-dioxygenase (IDO1) expression in breast cancer cells by activated CD8+ T cells is controlled by DNA promoter methylation

Gu F, Noonpalle SK K, Lee E-J, Choi J-H, Shull AY Y, Pei L, Sreekumar A, Ambs S and Shi H. Baylor College of Medicine, Houston, TX; Georgia Regents University Cancer Center, Augusta, GA; Georgia Regents University, Augusta, GA and Georgia Regents University, Augusta, GA.

Body: Tumor infiltrating lymphocytes (TILs) play a critical role in regulating the immunomodulatory properties of triple negative breast cancer (TNBC). However, the specific adaptations that TNBC tumors undergo when challenged by lymphocyte infiltration remain unclear. In order to address this gap in knowledge, we conducted an immuno-phenotype comparison using mRNA sequencing between the TNBC cell line MDA-MB-231 and the luminal breast cancer cell line MCF7 after both were co-cultured with activated human T-cells. Although the cytokine-induced immune signature of the two cell lines were similar, MDA-MB-231 cells were able to transcribe the tryptophan catabolizing enzyme *IDO1* at a significantly higher level than MCF7 cells. Stimulation with IFN γ was able to differentially induce IDO protein expression and enzymatic activity in ER- cell lines compared to ER+ cell lines, though no differences were observed in upstream JAK/STAT1 signaling or *IDO1* mRNA stability between the two cell lines. Further experiments showed that treatment with the demethylating agent 5-aza-deoxycytidine was able to reverse suppression of *IDO1* expression in MCF7 cells, suggesting that DNA methylation serves as a potential determinant in *IDO1* induction. Analysis of TCGA and other previously published breast cancer datasets revealed subtype-specific mRNA and promoter methylation differences in *IDO1*, with TNBC/basal-like subtypes exhibiting lower promoter methylation and higher mRNA expression than ER+/luminal subtypes. Bisulfite pyrosequencing validated the subtype-specific association of decreased promoter methylation with increased *IDO1* expression in breast cancer cell lines and an independent cohort of primary breast tumors. In addition, decreased *IDO1* promoter methylation and elevated *IDO1* expression in basal-like breast tumors was found to be associated with increased levels of kynurenine, the metabolic product of *IDO1*, as well as higher numbers of CD8+ TILs. Furthermore, high kynurenine levels in breast tumors were associated with worse patient survival. Taken together, these findings suggest that subtype-specific *IDO1* promoter methylation regulates the ability of breast tumors to escape from antitumor immune responses driven by CD8+ TILs and could be used as a predictive biomarker for IDO inhibitor-based immunotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-02-09

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-03-01

Title: A combination of dual inhibition in HER2-network by T-DM1 and GDC-0980 provides maximal antitumor efficacy in preclinical model of HER2+ breast cancer

De PK K, Carlson JH H, Sun Y, Lin X, Friedman L, Dey N and Leyland-Jones B. Avera Center for Precision Oncology, Sioux Falls, SD and Genentech Inc., SF, CA.

Body: Background: *PIK3CA* mutation is associated with a lower pCR rate in primary HER2+ breast cancer (BC) treated with trastuzumab and lapatinib in addition to chemotherapy (from five clinical trials, *PMID: 27177864*). The BOLERO-1 study showed that the efficacy of a combination of mTOR inhibitor (everolimus) plus trastuzumab (T) and paclitaxel was not very efficacious with HER2+ advanced BC patients except for HER2+/ER- BC patients (*PMID: 26092818*). In the same line, BOLERO 3 trial data showed the same combination (T + everolimus + vinorelbine) is also not efficacious in T-resistant, HER2+ advanced breast cancer women (median PFS 7 months with everolimus and 5.78 months with placebo, HR: 0.78) (*PMID: 24742739*). T-DM1 does not have typical adverse events of chemotherapy. Therefore, there has been interest in combining it with other targeted agent. Here we tested the efficacy of a combination of T-DM1 plus GDC-0980 (a dual PI3K/mTOR inhibitor) in HER2+/T-resistant BC cell lines *in vitro* and *in vivo*. **Methodology:** Here we have studied the *in vitro* and *in vivo* effects of GDC-0980 along with T-DM1 in HER2+/T-sensitive (BT474), HER2+/T-resistant (BT474HerR), and HER2+/PIK3CA (HCC1954, MDA-MB453) mutated models. We assessed *in vitro* anti-proliferative, pro-apoptotic and activation status of the PI3K-AKT-mTOR signaling pathway following the combination of GDC-0980 plus T-DM1 in HER2+ BC cell lines. We next evaluated the impact of GDC-0980 plus T-DM1 on tumor growth and angiogenesis using xenograft models. **Results:** 1) GDC-0980 inhibited downstream activation of the PI3K-mTOR signaling pathway effectors, p-AKT (Ser473, The308), p-P70S6K, p-S6RP and p-4EBP1, and this inhibition was more pronounced when GDC-0980 was combined with T-DM1, 2) similarly the anti-proliferative activity of a combination of GDC-0980 plus T-DM1 was significantly higher by 3D-ON-TOP clonogenic assay following heregulin stimulation, 3) consistent with anti-proliferative effects of GDC-0980, the proportion of cells in the G1 phase of the cell cycle increased in HER2+ cell lines with a concomitant decrease in the S phase of their treatment with GDC-0980, 4) the initiation of apoptotic activity (annexin V) of GDC-0980 was significantly superior to that of an allosteric inhibitor of mTOR, RAD001. GDC-0980 also induced apoptotic markers like cleaved CASPASE3, cleaved PARP1, BIM in HER2+ BC cells and 5) a combination of GDC-0980 plus T-DM1 significantly blocked tumor growth to tumor regression in the HER2+/T-sensitive, HER2+/T-resistant and HER2+/PIK3CA mutated BC xenograft models. Along with its anti-tumor effect, this combination effectively decreased tumor angiogenesis (tumor micro-vessel density via CD31 staining). **Conclusions:** A combination of GDC-0980 plus T-DM1 significantly blocked *in vitro* and *in vivo* HER2+ breast tumor cells growth irrespective of *PIK3CA* mutation status. This strategy warrants further clinical investigation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-03-02

Title: Chronic CXCL12 exposure induces a metastatic phenotype in ER-positive breast cancer cells through transcriptional reprogramming

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Body: The chemokine CXCL12 is transcriptionally activated by estrogen in estrogen receptor (ER)-positive breast cancer cells. We have found that CXCL12 signaling is essential to maintain the long-term growth of ER-positive breast cancer cells and promotes cancer cell growth in the absence of estrogen. Chronic blockade of CXCL12 signaling with AMD3100, an inhibitor of CXCL12 receptor CXCR4, causes cell death in these cells. Chronic exposure to CXCL12 reprograms ER-positive breast cancer cells through genome-wide transcriptional changes and activates numerous signaling pathways including EMT and the inflammatory response. Many ER target genes are activated in CXCL12-reprogrammed cells even in the absence of estrogen which leads to the diminished estrogen modulated transcription in these cells. These cells also show enhanced signaling via TGF β , EGFR and Rac1 pathways, rendering these cells more sensitive to the CDK7 inhibitor, THZ1, and to drug combinations of THZ1 with the EGFR inhibitor Gefitinib or the RAC1 inhibitor EHT 1864. Furthermore, CXCL12-reprogrammed ER-positive breast cancer cells become more motile *in vitro* and display a metastatic phenotype in a mouse model. The lung-tropic phenotype of CXCL12-reprogrammed MCF-7 cells could be explained by increased expression of integrins and pro-inflammatory signaling molecules. Our novel finding of chronic CXCL12 action on ER-positive breast cancer cells suggests a mechanism by which the interaction between stromal and tumor cells leads to increased breast tumor metastatic potential.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-03-03

Title: Tropomyosin-related kinase A is overexpressed in HER2-positive breast cancers

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Body: The neurotrophin tyrosine kinase receptor TrkA (NTRK1) contributes to breast cancer cell invasion and metastasis, but its clinicopathological significance in breast tumours is unclear. In this study, TrkA immunohistochemistry was performed in a cohort of 366 breast tumours including ductal carcinomas in situ (DCIS), invasive ductal carcinomas (IDC), and invasive lobular carcinomas (ILC). TrkA protein was detected in both cancer and myoepithelial cells and was preferentially associated with IDC (39% of cases) as compared to DCIS (24% of cases) ($p < 0.0001$) and ILC (20% of cases) ($p < 0.0001$). There was a linear association between TrkA expression and lymph node invasion ($p = 0.0052$), confirming the involvement of TrkA in the metastatic potential of breast cancer cells. Interestingly, TrkA expression was significantly increased in tumours positive for the human epidermal growth factor receptor-2 (HER2). TrkA was detected in 48% of HER2-positive tumours compared to only 25% of HER2-negative tumours ($p < 0.0001$). When considering molecular subtypes of breast cancer, HER2+ and luminal B tumours were associated with higher TrkA levels ($p = 0.006$). In Western-blotting, TrkA immunoreactivity was observed at 140 kDa and 180 kDa, with higher levels of TrkA in HER2-positive as compared to HER2-negative breast cancer cell lines. TrkA tyrosine kinase activity in breast cancer cell lines was inhibited by various pharmacological inhibitors and the impact on cell growth was investigated. Together, these data reveal the association between TrkA and HER2 protein expression in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-04-01

Title: Engineering mesenchymal stem cells(MSCs) to secrete tumour-suppressing exosomes for breast cancer therapy

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Body: Introduction: Mesenchymal Stem Cells(MSCs) are multipotent stromal cells that are known to engraft into tumours, raising their potential as tumour-targeted delivery vehicles. MSCs secrete tiny vesicles known as exosomes, which contain genetic material including microRNAs, and are effectively taken up by recipient cells. This study aimed to characterise a tumour-suppressing microRNA, miR-379, and engineer MSCs to secrete exosomes enriched with the microRNA.

Methods: The mechanism of action of miR-379 In Vivo was determined through lentivirus-mediated upregulation of miR-379 in breast tumours, and analysis of changes in tumour angiogenesis, proliferation and progression. Subsequently, MSCs were engineered with lenti-379 and any impact on MSC migration, proliferation and morphology was assessed. MSC-secreted exosomes were isolated and characterised using Transmission Electron Microscopy(TEM) and Western Blot. The exosomal microRNA content was analysed by RQ-PCR, and transfer between cell populations visualised using confocal microscopy.

Results: While elevated miR-379 expression did not impact tumour size In Vivo, an increase in tumour necrosis and decrease in invasion was observed. MSCs were successfully transduced with miR-379, resulting in a distinct change in cell morphology. Despite this, MSC-379 cells maintained inherent tumour-targeted migratory capacity, with no impact on proliferation observed. MSC-379 derived exosomes were 30-120nm in size and expressed the exosome-associated protein CD63. A 5-fold increase in miR-379 was observed in engineered exosomes. Successful transfer of RFP-labelled MSC-derived exosomes to breast cancer cells was visualised using confocal microscopy.

Conclusion: Engineering tumour-targeted MSCs to secrete exosomes enriched with miR-379 holds exciting potential as a novel therapy for breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-05-01

Title: Low BRCA2 levels cause epigenetic activation of growth adaptive pathways without inducing recurrent genomic mutations

Gruber JJ J and Snyder M. Stanford University, Palo Alto, CA.

Body: The development of therapeutics to prevent cancer onset is hindered by a lack of robust cell systems to study the earliest steps of malignant transformation. Significant barriers to studying cancer-initiating events include intrinsic cellular pathways to prevent cell proliferation in the setting of activating oncogenes or genomic instability. To circumvent this limitation, we studied *in vitro* transient depletion of breast cancer tumor suppressor genes followed by selective growth conditions to enrich for *de novo* mutations or stable epigenetic alterations that can confer a growth advantage to non-transformed breast epithelial cells. We report that transient BRCA2 depletion, but not depletion of other breast cancer genes, leads to a selective growth advantage in EGF-free media. This is due to a failure of BRCA2 inactivated cells to enact a mesenchymal-to-epithelial transition upon withdrawal of EGF. Genomic profiling and biochemical approaches indicate that transient loss of BRCA2 induces chromatin remodeling in concert with NF- κ B signaling and histone H2B deacetylation to repress genes associated with the epithelial state. Notably, this process did not appear to require recurrent driver mutations, nor DNA methylation changes, but was dependent on ATR signaling. Overall, our work suggests that transiently low BRCA2 levels are sufficient to induce aberrant cell proliferation under certain conditions and that targeting epigenetic modifiers may be an approach to prevent BRCA2-induced malignancy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-05-02

Title: Breast cancer increased risk on women using combined oral contraceptive

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Body: The most controversial aspect in relation to oral hormonal contraception is the risk of developing breast cancer. Knowledge of the factors that influence the proliferation of normal breast epithelium is essential to understanding carcinogenesis and proliferative activity can be assessed by various techniques, including the immunohistochemical analysis of Ki 67. In this work, we aim to compare the overall proliferative activity of breast after 3 cycles of combined oral contraceptive with a natural cycle and with that presented after only one cycle. Were selected 65 women attended in the period July 2006 to February 2007. These underwent excision of breast lump and breast tissue macroscopically regular adjacent to the node, lying this at least 1 cm. The patients were divided into two groups: group A, consisting of 20 women using three cycles of combined oral contraceptive consisting of 150 ug levonorgestrel and 30 ug of ethinyl estradiol, and the group B consisting of 35 women with natural cycles eumenorrheic , ie not used any hormonal medication. In the fourth month after initiation of use of the ACO, the group A patients were divided into four groups according to the use of the contraceptive. The A1 group (5 patients) in the first week; A2 (5 patients) in the second week; A3 (5 patients) in the third week and the A4 (5 patients), the break of the week . Results: The highest levels of Ki67 count occurred in the third (16.65%) and fourth week (16.56 %) similar to one another. Additionally, it is noted that the average pause week count was greater than the first week (10.25%) and of the second week (8.52%), which in turn were not distinct. When comparing the use of 3 cycles with 1 cycle, we found that there was no difference in Ki67 count levels by the time the first two weeks. However, note that the Ki67 levels were higher in women who used contraceptives for longer both in the third week as the week's break.

Measures summary score * Ki-67 (%) by contraceptive use time over the weeks

Contraception use	Media	Minimum	Maximus	Number	p
Week 1					0,180
1 month use	7,02	2,44	18,38	11	
3 monthas use	10,42	5,61	18,34	5	
Week 2					0,180
1 month use	5,46	1,13	13,17	11	
3 months use	8,52	5,66	10,20	5	
Week 3					0,005
1 month use	4,77	2,04	11,84	11	
3 months use	16,66	5,52	29,30	5	
Week 4					0,001
1 month use	4,44	0,20	9,78	9	
3 months use	16,69	11,29	22,91	5	

In conclusion, the proliferative activity of artificial cycle was significantly higher than the natural cycle

Measures summary score * Ki-67 (%) by use of OHC for three months over the weeks and the natural cycle

	Media	Minimum	Maximun	Number	p
Week 1					0,003
3 months use	10,42	5,61	18,34	5	
natural	1,10	0,09	2,44	9	
Week 2					0,010
3 months use	8,52	5,66	10,20	5	
Natural	3,89	0,70	9,50	10	
Week 3					0,003
3 months use	16,66	5,52	29,30	5	
Natural	2,72	0,30	8,56	10	
Week 4					0,004
3 months use	16,69	11,29	22,91	5	
Natural	5,39	1,20	15,55	11	

, and after three cycles, showed higher rates of proliferation in the third and fourth weeks of the menstrual cycle, representing increased carcinogenesis and possible increased breast cancer risk. More studies including compensatory apoptosis must be considerer to confirm this hipotesis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-05-03

Title: Investigating the effects of low-dose mixtures of environmental contaminants on breast cancer initiation

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Body: The alarming increase in the incidence of breast cancer worldwide cannot be explained solely by hereditary factors and strong evidence suggests that a substantial risk can be attributed to extrinsic exposures. In fact, estimates show that 26.8% of all new breast cancers are related to lifestyle and environmental factors. While several lifestyle factors, such as alcohol and a high fat diet, have been identified as risk factors for breast cancer, still little is known about the contribution of environmental factors, such as environmental contaminants, to breast cancer risk. Research has shown that chemicals, such as plasticisers, pesticides and cosmetic additives cause effects in animal and cell-based models that promote breast cancer. However, many of these effects are only seen at levels of the chemicals that are much higher than those found in human tissues. Also, most studies examining links between exposure to individual chemicals and breast cancer in humans have produced unconvincing results. These observations often lead to the assumption that the concerns associated with the role of chemicals in breast cancer are unfounded, as their levels in tissues are not high enough to increase breast cancer risk. The problem with this assumption is that humans are exposed to low levels of large numbers of chemicals that could act together to increase breast cancer risk. We, and others, have demonstrated the importance of mixture effects *in vitro* and *in vivo*, however, this possibility has not been investigated in breast cancer.

Our work aims to address this question by studying the way chemicals act together at low levels to potentially cause breast cancer. We analysed a mixture of four ubiquitous chemicals, all combined at levels found in human tissues: the plasticiser Bisphenol A, the pesticide *o,p'*-DDT (although banned, it is still present in tissues), the cosmetic preservative propylparaben and the UV filter benzophenone-3. The effect of this mixture on the structure and behaviour of the breast epithelium was investigated in a three dimensional culture system that resembles the mammary tissue. Here, we observed that all chemicals induced a dose-dependent increase in the disruption of acinar structures, characterised by the formation of aberrant, highly proliferative and disorganised acini with filled lumen, resembling the events that occur during breast carcinogenesis. Interestingly, these effects were also observed when chemicals were combined at very low levels that, individually, produced no measurable effects. Finally, we observed that similar low-dose mixtures elicited changes in the epigenetic and genetic profile of acini, suggesting an epigenetic link between chemical exposure and cancer development.

The outcome of this work paves the way to better estimations of the realistic breast cancer risk in women and provides an essential framework to inform the development of practical solutions, guidance and advice to help reduce exposure of women.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-05-04

Title: Relationship between obesity and breast tumor pathology in a contemporary set of patients

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Body: Background: Obesity is a risk factor for breast cancer in postmenopausal women, and is associated with decreased survival regardless of menopausal status. This outcome disparity has been attributed to clinical factors such as delayed diagnosis, inadequate dosing with chemotherapeutic agents, and co-morbid conditions. Here, we evaluated the association between pathological characteristics and BMI in a cohort of patients enrolled in the Clinical Breast Care Project (CBCP) 2001-2013.

Methods: All female patients with invasive breast cancer who had height and weight recorded at the time of diagnosis were identified from the CBCP database. BMI was calculated and patients categorized as normal/lean (≤ 24.9 kg/m²), overweight (25-29.9 kg/m²), or obese (≥ 30.0 kg/m²). Chi-square univariate analysis was performed to determine statistical significance followed by multivariate logistic regression. Statistical significance was defined as $p < 0.05$. Survival analysis was done using Cox proportional hazard regression models.

Results: Of the 1,705 patients, 32.4% were normal/lean, 31.6% were overweight and 36.0% were obese. Univariate analysis revealed that obese women were significantly more likely to be older at age at diagnosis, African American, menopausal and treated at Joyce Murtha Breast Care Center, a rural civilian hospital in western-central PA. These factors were thus include in multivariate analysis which showed that obese patients were at an increased risk relative to normal/lean patients of having a higher tumor stage (OR = 1.32, 95% CI [1.043-1.681], $p = 0.021$), size (OR = 1.42, 95% CI [1.091-1.857], $p = 0.009$), and grade (OR = 1.37, 95% CI [1.085-1.718], $p = 0.008$) but at decreased risk of PR- tumors (OR=0.57, 95% CI [0.394-0.837], $p = 0.004$). Overweight patients did not differ significantly from either normal weight or obese patients. Neither breast cancer-specific nor overall survival differed significantly between groups.

Discussion: Higher tumor stage and size may reflect delayed detection in obese women. In contrast, tumor grade and PR status likely reflect underlying biological differences driven by increased adiposity in the breasts of obese women. Despite these differences in pathology, survival did not differ by BMI status, suggesting that factors such as suboptimal dosing may not be relevant in current patient care.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-05-05

Title: The expression of lysyl oxidase and fibrotic focus is related to inflammation in breast cancer

Park SH Hwan, Jeong YJ Ju, Bong JG Gu and Oh HK Kyu. Daegu Catholic University Medical Center, Daegu, Republic of Korea.

Body: Background: Lysyl oxidase (LOX) is an extracellular matrix enzyme that catalyzes the cross-linking of collagens or elastin. Our hypothesis is that LOX contributes to the formation of a fibrotic focus (FF), which is related to inflammation in breast carcinogenesis.

In this study, we analyzed the association between the expression LOXs and FF, and investigated prognostic significance in breast cancer.

Methods: Tissue microarrays were constructed from the specimens of 444 patients with primary invasive breast cancer. Immunohistochemical staining for LOX, LOXlike (LOXL)-, LOXL-2 and LOXL-3 was performed. The status of FF within the tumor was assessed. The number of CD4+ T cells, CD8+ T cells, CD68+ macrophages was counted, and intratumoral and peritumoral lymphocyte infiltration were evaluated.

The clinicopathologic characteristics of the patients were analyzed.

Result: The percentage of positive FF was 39.2% and positive rate of LOX expression was 50% in primary breast cancer tissues. FF was found to be significantly associated with intratumoral and peritumoral inflammation, lymph node metastasis, high histologic grade, larger tumor size. LOX was associated with intratumoral and peritumoral inflammation, CD8+ T cells and menopausal status. LOXL-3 was significantly associated with positive expression of ER and PR, and molecular subtype.

Conclusions: FF and the expression of LOX were associated with inflammation in breast cancer in this study. Our results suggest that LOXs may contribute to the formation of a FF indirectly in relation with inflammation in breast cancer. Further studies are needed to clarify the role of LOXs, FF and inflammation in tumorigenesis and prognostic value of them in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-06-01

Title: High-grade human cytomegalovirus IEA is associated with expression of COX-2 and 5-LO in human breast cancer samples

Costa H, Touma J, Davoudi B, Geisler J, Vetvik K, Söderberg Naucner C and Rahbar A. Karolinska Institute, Sweden.

Body: Background: The role of cyclooxygenase-2 (COX-2) in breast cancer development and progression has been supported by an increasing number of studies that show the overexpression of COX-2 in all the stages of the disease but in particular, in the metastatic phase. Besides COX-2 and its derived metabolites, 5-lipoxygenase (5-LO) and leukotrienes have also been associated with cancer progression. Human Cytomegalovirus (HCMV) detection in samples from primary BC, sentinel lymph nodes and brain metastases obtained from breast cancer patients' suggests that viral infection may also have a critical role in the development of breast cancer metastasis. Interestingly, *in vitro* studies showed that HCMV infections induce COX-2 in human fibroblasts, which augments viral replication through a prostaglandin dependent pathway. Thus, our main objective was to investigate whether there is a correlation between HCMV infection and overexpression of COX-2 and 5-LO in breast cancer. If so, HCMV could be an important additional target for breast cancer treatment.

Material and Methods: Paraffin embedded breast cancer biopsies (n=48), ductal carcinoma in-situ (DCIS, n=14) and adjacent, benign breast tissue samples (n=29) were retrospectively examined for HCMV-immediate early (IE), HCMV-Late (LA) proteins, COX2 and 5LO by using immunohistochemical techniques established in our laboratory. Clinical data were available from the patients' hospital files provided from the departments of oncology and pathology at Akershus University Hospital, Norway. All patients underwent direct surgery in 2011. All patients received standard adjuvant treatment according to the Norwegian guidelines. For *in vitro* studies, breast cancer cell lines (MCF-7, MB-MDA-231 and SKBR3) were infected with HCMV VR1814 strain and levels of COX-2 and 5-LO were determined by qPCR and western blot and immunofluorescence.

Results: High levels of COX-2, 5-LO and HCMV-IE were detected mainly in breast cancer samples. High grade HCMV-IE (defined as >50% positive cells in the tumor tissues) was detected in 72% of infiltrating BC and in 28% of DCIS, but it was detected only in 7% of benign, adjacent breast tissue samples. Similarly, high grade COX-2 and 5-LO were detected in 58% and 53% of BC, in 21% and 8% of DCIS, and in 4% and 7% of benign, adjacent breast tissue samples, respectively. We found a statistically significant positive correlation for the levels of HCMV-IE and COX-2 ($p=0.001$) as well as for HCMV-IE and 5-LO ($p=0.0002$) in infiltrating breast cancer. Furthermore, induction of COX-2 and 5-LO was confirmed in breast cancer cell lines following infection with HCMV was shown at both mRNA and protein level.

Conclusion: Our findings confirm a positive correlation of HCMV-IE protein synthesis and overexpression of COX-2 and 5-LO in infiltrating breast cancer, DCIS and benign, adjacent breast tissue samples, which is consistent with the up-regulation of these enzymes in breast cancer cells infected with HCMV. These results suggest that the inflammation driven by COX-2 and 5-LO in human breast cancer might be induced by HCMV infections and lead to tumor progression. Thus, anti-viral therapy should be considered as an additional experimental treatment in selected breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-06-02

Title: Low expression levels of hormone receptors (ER- α and PGR) in human breast cancer samples is significantly associated with high-grade human cytomegalovirus-IEA

Rahbar A, Touma J, Costa H, Davoudi B, Vetvik K, Geisler J and Söderberg Naucner C. Karolinska Institute, Sweden.

Body: Background: Breast cancer (BC) is the most common type of malignancy and second leading cause of cancer deaths in women worldwide. While contemporary breast cancer therapy allows many patients with localized breast cancer to be cured for their disease, subgroups experience non-curable distant metastasis. In addition, majority of BC cases are considered to be "sporadic", with unknown underlying mechanisms. Thus, it is of major importance to investigate alternative factors that may initiate or promote human breast cancer. Recently, human cytomegalovirus (HCMV) has been detected in samples from primary BC, sentinel lymph nodes and brain metastases obtained from breast cancer patients. However, the oncomodulatory role of HCMV in BC is unknown. Accordingly, the purpose of this study was to elucidate possible correlations between expression of HCMV proteins and established histopathological markers (ER- α , PgR, HER2 etc.) in human breast cancer tissues. **Material and Methods:** Paraffin embedded breast cancer biopsies (n=62), ductal carcinoma in-situ (DCIS, n=18) and adjacent, benign breast tissue samples (n=42) were retrospectively examined for HCMV-immediate early (IE) and late (LA) proteins by using immunohistochemical techniques. Clinical data were available from the patients' hospital files provided from the departments of oncology and pathology at Akershus University Hospital, Norway. All patients underwent direct surgery in 2011. The median age at time of surgery was 55 years. All patients received standard adjuvant treatment according to the Norwegian guidelines. **Results:** HCMV-IE was detected at different levels in all BC cases, DCIS and benign breast tissue samples. Interestingly, high grade HCMV-IE (defined as >50% positive cells in the tumor tissues) was detected in 77% of infiltrating BC and in 39% of DCIS and merely in 7% benign breast tissue samples. High grade HCMV-IE was detected in 40%, 76% and 83% of BC patients with >50-90%, >10-50% and <10% tumor cells expressing PgR in their tumors, respectively ($p=0.003$). All BC samples with negative or low (0-10%) positive staining for estrogen receptors (ER- α) showed high-grade HCMV-IE staining. In subgroups of patients with increasingly positive staining for ER- α between 10-50% and >50-90% of tumor cells, high HCMV-IE was found in 86% and 74%, respectively ($p=0.02$). A trend but no significant correlation was found between high-grade HCMV-IE and HER2 negativity ($p=0.09$). In contrast, High grade HCMV-LA proteins were detected in 5%, 11% and 3% of adjacent benign breast samples, DCIS and infiltrating BC, only. **Conclusion:** Our findings demonstrate that HCMV-IE but not HCMV-LA proteins are frequently detected in samples obtained from infiltrating BC and DCIS. Although the role of HCMV in carcinogenesis of BC is not defined, our findings suggest a negative correlation between high grade HCMV-IE and hormone receptors in general. All in all, our findings may indicate a possible oncomodulatory role of HCMV-IE in human BC, hampering the expression of hormone receptors and forcing the BC cells to a more aggressive phenotype. The effects of HCMV-induced proteins in human breast cancer cells should be investigated further.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-01

Title: Development of a Prosigna® (PAM50)-based classifier for the selection of advanced triple negative breast cancer (TNBC) patients for treatment with enzalutamide

Danaher P, Skewis L, Mashadi-Hosseini A, Ram N, Gowen-MacDonald J, Harris E, Ferree S and Buckingham W. NanoString Technologies, Inc., Seattle, WA.

Body: Background: Enzalutamide is an orally administered androgen receptor (AR) inhibitor approved by the FDA for use in men with metastatic castrate-resistant prostate cancer. A recent phase II study of enzalutamide in patients with advanced, AR positive, TNBC (NCT01889238) demonstrated significant improvements in both PFS and OS for patients whose tumors exhibited a gene expression (Gx) profile enriched in AR signaling and luminal biology. A PAM50-based signature was developed from the phase 2 study which used next generation RNA sequencing (NGS) to identify patients likely to respond to enzalutamide. We transitioned the test to the NanoString (NS) nCounter® Analysis System using Prosigna reagents to support clinical validation in a phase 3 trial. Here we describe the development and analytical performance of the NanoString Androgen Gene Expression Profiling Assay-1 (NS-AR-01). **Methods:** The NS-AR-01 algorithm coefficients were calibrated from the Predict AR algorithm by testing FFPE tumor tissue from patients who were pre-screened but not enrolled in the phase II study with both platforms (NGS and NS). Three unique algorithms were developed and subsequently challenged with an independent sample set with NGS data to provide an unbiased evaluation of the concordance of the platforms. A pre-specified clinical accuracy verification study was performed through prediction of NS-AR-01 scores from the NGS Gx data from the patients included in the phase 2 study efficacy analysis. The final NS-AR-01 algorithm was selected based on performance in the clinical accuracy verification. The final NS-AR-01 algorithm was evaluated in the 118 patients included in the ITT analysis, as well as those treated with 0-1 lines of prior therapy. The analytical performance of the assay was characterized by testing precision from RNA, reproducibility from FFPE tissue, sensitivity to RNA input amounts, and the impact of common interferences. **Results:** All three algorithm translations met the pre-specified clinical accuracy verification acceptance criteria. The final NS-AR-01 algorithm generated a hazard ratio most similar to that observed from the NGS algorithm. The total standard deviation when testing multiple FFPE sections from the same block was < 1.5% of the score range with an empirical concordance rate of 100% for biomarker status. The range of RNA input specified for Prosigna was successfully verified for NS-AR-01 (125ng–500ng total RNA). The assay was demonstrated to be robust to common interferences including non-tumor tissue. **Conclusions:** Based on these results, NS-AR-01 is an accurate, precise, and robust assay for the identification of advanced TNBC patients who may respond to treatment with enzalutamide. The assay is well suited to clinical applications, and its ability to identify responders to enzalutamide will be evaluated in future investigational studies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-02

Title: CDK4 phosphorylation status and corresponding gene expression profile predict sensitivity to Palbociclib

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Body: Although the specific CDK4/6 inhibitor PD0332991 (Palbociclib) was recently approved by the FDA to treat advanced ER+ breast tumors, there is yet no reliable sensitivity prediction tool. Cyclin D-CDK4/6 are the first CDK complexes to be activated in G1 phase in response to oncogenic pathways. They phosphorylate and inactivate the central cell cycle/tumor suppressor pRb. CDK4 activity requires its binding to a cyclin D (*CCND1-3* genes) with which INK4 CDK4 inhibitors such as p16 (*CDKN2A-D* genes) compete. Although the assembly of the CDK4-cyclin D complexes was considered to be the main level of CDK4 activity control, we have shown that the activating T172-phosphorylation of CDK4 is actually the central rate-limiting event that initiates the cell cycle decision and signals the presence of active CDK4.

Here, using 2D-gel electrophoresis to separate the modified forms of CDK4, we found in breast cancer cell lines that only the CDK4 T172-phosphorylation correlates with the sensitivity to PD0332991. The only exception was in the rare case of combined CCNE1 amplification and CDKN2A loss wherein combination of PD0332991 with a CDK2 inhibitor is required to block entry in the cell cycle. Additionally, three types of CDK4 modification profile were identified by 2D-gel electrophoresis in 56 breast tumors. In the first profile, the phosphorylated CDK4 was undetectable as in normal breast samples despite a high KI67 index. In the second and third profiles, the CDK4 phosphorylation was detectable and its intensity was either above or below 90% of the intensity of a second yet unidentified form of CDK4, respectively. The proportions of these profiles differ among breast tumors according to their clinic-pathological characteristics, molecular subtypes and risk. Finally, we identified a 11-gene expression signature that faithfully predicted the CDK4 modification profiles of breast tumors and cell lines (concordance rates of 84% and 100% in the 56 analyzed breast tumor samples or cell lines respectively). All three CDK4 modification profiles were evaluated in a merged independent dataset of 4034 published gene expression profiles. In these 4034 patients, 70% of triple-negative tumors, 18% of HER2-positive tumors and 5% of ER-positive tumors were predicted to have the first CDK4 profile wherein CDK4 phosphorylation is undetectable and to be completely unresponsive to CDK4 inhibitors. The phosphorylated CDK4 was predicted to be the major modified form in 26% of triple-negative tumors, 48% of HER2- positive tumors and 56% of ER-positive tumors. These patients should benefit the most from treatment with CDK4 inhibitors. Therefore, prediction of the CDK4 modification profile may allow extending treatment with Palbociclib to presently ineligible patients. As tumors with the third CDK4 modification profile generally present low grade and low OncotypeDX risks, the added value of including CDK4 inhibitors in their treatment compared to surgery and hormone therapy alone is questionable.

In conclusion, we identified CDK4 phosphorylation as the most direct biomarker of CDK4 inhibitor sensitivity in breast cancer and developed a promising 11-gene based surrogate marker to guide their use in the clinic.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-03

Title: Broken promise of liquid biopsy: Plasma DNA does not accurately reflect tumor DNA in metastatic breast cancer

Shee K, Chamberlin MD D, Varn FS S, Bean JR R, Marotti JD D, Wells WA A, Trask HW W, Hamilton JS S, West RJ J, Kaufman PA A, Schwartz GN N, Gemery JM M, McNulty NJ J, Tsapakos MJ J, Barth RJ J, Arrick BA A, Gui J, Cheng C and Miller TW W. Geisel School of Medicine at Dartmouth, Hanover, NH and Dartmouth Hitchcock Medical Center, Lebanon, NH.

Body: Background: Circulating tumor DNA in plasma may present a minimally invasive approach to identify tumor-derived mutations that could be used to inform the selection of targeted therapies for individual patients, particularly in cases of metastatic disease where biopsy is often difficult. We hypothesized that plasma DNA will genetically reflect DNA derived from multiple tumors in patients with metastatic breast cancer. To test this hypothesis and assess the utility of plasma DNA obtained as a “liquid biopsy” for precision medicine, we sought to determine whether massively parallel sequencing of plasma DNA is a reliable surrogate for sequencing of DNA from tissue biopsies in patients with metastatic breast cancer.

Methods: Blood samples were obtained from 7 patients with multiple advanced breast cancer lesions (recurrent breast and metastatic tumors), and tumor specimens were obtained thereafter by biopsy or surgical excision. DNA extracted from plasma, buffy coat of blood, and tumor tissues was used for probe-directed capture of all exons in 196 genes followed by massively parallel sequencing with an average coverage of 3000x for plasma DNA. Tumor and plasma DNA sequences were bioinformatically compared to buffy coat controls, and high-confidence somatic mutations were called. One patient with extensive metastatic disease was evaluated in further detail to study the contribution of different tumors to the overall plasma DNA pool. In this patient, 9 metastatic tumors were sampled in an axillary lymph node, heart, kidney (2), liver, omentum (3), and ovary by biopsy or at autopsy.

Results: Mutations were found in plasma that were represented in one or more tumors in each patient. Three classes of mutations were discovered: 1) mutations overlapping between both plasma and tumors (e.g., *TP53* p.R273C and *SRC* p.E527K); 2) mutations found in plasma but not tumors (e.g., *AKT* p.E17K and multiple known and novel *ESR1* mutations); 3) mutations found in tumors but not plasma (e.g., *PIK3CA* p.H1047R, p.D350G, and p.N345K). The presence of mutations in each of these classes was validated in plasma and/or tumors using mutation-specific droplet digital PCR (ddPCR).

In the patient with extensive metastatic disease, DNA sequencing revealed heterogeneity of tumor contribution to plasma DNA, with some tumors better represented than others. No correlation was found between tumor size (measured by CT scan) and mutational burden in plasma. Interestingly, a significant correlation was found between blood perfusion to the organ where the tumor resides and mutational burden in plasma, with the greatest tumor contribution coming from the heart metastasis (Pearson's $r = 0.835$, $p=0.039$).

Conclusions: Plasma DNA sequencing adds a layer of depth to sequencing analysis of tumor biopsy samples, and serves to both confirm tumor-derived mutations as well as detect new mutations. However, plasma DNA profiling does not comprehensively reflect the mutational profiles of tumors in patients with metastatic breast cancer, and thus is unlikely to serve as a surrogate for tumor biopsy as a source of DNA for genetic profiling. Furthermore, plasma DNA contains many mutations not found in tumors, which will confound treatment decision-making and precision medicine.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-04

Title: Comprehensive genomic profiling to assess tumor mutation burden in >8,000 breast cancer cases: Implications for immunotherapy

Frampton GM M, Connelly C, Fabrizio D, Miller VA A and Stephens PJ J. Foundation Medicine, Cambridge, MA.

Body: Background

Breast cancer presents a management challenge, particularly when cytotoxic, hormone and anti-HER2 (ERBB2) therapies are not indicated or have failed. Tumor mutational burden (TMB) is linked to predicted benefit from immune checkpoint inhibitors in several types of advanced cancers, but its effectiveness in breast cancer has not yet been accurately assessed. Using comprehensive genomic profiling (CGP) we assessed the relationship between TMB, histologic sub-type, and clinically relevant genomic alterations in breast cancer samples in the course of routine clinical care.

Methods

DNA was extracted from 40 microns of FFPE sections from patients with breast cancer. CGP was performed on hybridization-captured, adapter ligation based libraries to a mean coverage depth of greater than 500x for up to 405 cancer-related genes. TMB was characterized as the number of somatic coding base substitution and indel mutations per megabase of genome assessed. Cases were stratified by patient age, histologic sub-type, and presence or absence of therapeutically relevant genomic alterations. The vast majority of samples were from patients with advanced disease.

Results

The genomic profiles from a total of 8302 breast cancer samples from unique patients were assessed. Median patient age was 54 years. 10th, 25th, median, 75th, and 90th percentiles of TMB were 1.3, 2.5, 4.5, 6.3, and 10.1 mutations per megabase of coding genome. 210 cases (2.6%) had high TMB of ≥ 20 mutations per megabase. High TMB was found more frequently in breast invasive lobular carcinoma (24/437, 5.5%) than in breast invasive ductal carcinoma (56/3694, 1.5%) or breast carcinoma, sub-type not otherwise specified (124/3754, 3.3%). Of the 25 most frequently altered genes in this series (Table 1) *CDH1*, *ARID1A* and *RB1* were >3x more likely to be mutated in the high TMB cohort. Cases with high TMB had higher median age (62 years) than the cohort overall.

Gene	Alteration rate (High TMB, n=210)	Alteration rate (other TMB, n=7993)	Ratio
TP53	66.7%	55.9%	1.19
PIK3CA	60.0%	31.4%	1.91
MYC	16.2%	24.0%	.68
CCND1	16.2%	17.5%	.93
FGF19	14.9%	16.3%	.91
FGF4	13.4%	15.9%	.84
FGF3	13.9%	15.9%	.87
ZNF703	13.9%	14.7%	.95
FGFR1	14.3%	14.1%	1.01
PTEN	17.6%	12.4%	1.42
ERBB2	15.7%	12.2%	1.29
MYST3	8.9%	10.0%	.89
ESR1	10.5%	9.7%	1.08
CDH1	28.1%	9.1%	3.09
GATA3	7.9%	9.3%	.85

MCL1	4.3%	9.0%	.48
ZNF217	7.9%	7.8%	1.01
RB1	12.9%	7.1%	1.82
CDKN2A	5.2%	6.6%	.79
EMSY	5.9%	6.6%	.89
ARID1A	21.9%	5.7%	3.84
GNAS	6.7%	5.7%	1.18
LYN	5.1%	5.7%	.89
NF1	16.7%	5.3%	3.15
AURKA	5.7%	5.2%	1.10

Conclusions

CGP in the course of clinical care can be used to assess TMB in breast cancer. Cases with high TMB were relatively rare in breast cancer, as compared to other tumor types in which immunotherapies have been most effective. Nevertheless, a meaningful subset of breast cancer patients have high TMB and may be more likely to respond to immunotherapy treatments. Incorporation of CGP into ongoing prospective immunotherapy trials and clinical practice is needed to refine these relationships. Further correlation to clinical outcomes will be investigated to assess the correlation between tumor mutation burden and response to immunotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-05

Title: PD-L1 expression and decreased tumor-infiltrating lymphocytes are associated with poor prognosis in patients with triple negative breast cancer

Mori H, Kubo M, Yamaguti R, Nishimura R, Osako T, Arima N, Okumura Y, Okido M, Yamada M, Kai M, Kishimoto J, Oda Y and Nakamura M. Graduate School of Medical Sciences, Kyushu University, Fukuoka City, Japan; Kurume Medical Center, Kurume City, Japan; Breast Center, Kumamoto Shinto General Hospital, Kumamoto City, Japan; Kumamoto Shinto General Hospital, Kumamoto City, Japan; Kumamoto City Hospital, Kumamoto City, Japan; Hamanomachi Hospital, Fukuoka City, Japan; Faculty of Medical Sciences, Kyushu University, Fukuoka City, Japan and Graduate School of Medical Sciences, Kyushu University, Fukuoka City, Japan.

Body: Background: Tumor microenvironment has been considered to have an active role in determining the aggressiveness of tumor cells. Recently, programmed cell death ligand-1 (PD-L1) expression or tumor-infiltrating lymphocytes (TILs) are known to be an important prognostic factor of breast cancer. However, the correlation of expression of PD-L1 and TILs still remains unclear. Triple-negative breast cancer (TNBC) is a heterogeneous tumor that encompasses many different subclasses. Further identification of these subclasses is necessary in order to predict prognosis and choose appropriate treatments. Our goal was to correlate PD-L1 expression with clinicopathological features including TILs by using a large cohort of TNBCs.

Patients and Methods: This study included 248 patients with primary TNBC who underwent resection without neoadjuvant chemotherapy at our three hospitals between January 2004 and December 2014. The tumor subtypes were routinely determined immunohistochemically by using resected specimens. IHC scoring for PD-L1 expression was defined in reference to that for HER2 expression. PD-L1 positivity was defined as both IHC 2+ and IHC 3+. Cases were defined as high if stromal TILs $\geq 50\%$ according to recommendations by the International TILs Working Group.

Results: Of the 248 TNBCs, PD-L1 were expressed as positive in 103 (41.5%) tumors, and TILs were highly present in 118 (47.6%) tumors. PD-L1 expression was significantly correlated with higher levels of TILs ($P < 0.0001$). There was no significant difference when the prognosis of the patients who had PD-L1-positive tumors was compared with that of the patients who had PD-L1-negative tumors ($P = 0.56$ in recurrence free survival [RFS] and $P = 0.13$ in overall survival [OS]). Meanwhile, the patients with high-TILs tumors had longer OS, compared to the patients with low-TILs tumors ($P = 0.55$ in RFS and $P = 0.016$ in OS). The analysis in the cross effect between PD-L1 expression and TILs using cox proportional hazards model demonstrated that the PD-L1 expression and TILs are not independent factors ($P = 0.0018$ in RFS and $P = 0.015$ in OS). The PD-L1-positive group with low-TILs had significantly shorter survival than the PD-L1-positive group with high-TILs (hazard ratio [HR] = 4.7, 95% confidence interval [CI] 1.6–12.7, $P = 0.0067$ in RFS; HR = 8.4, 95%CI 2.3-30.3, $P = 0.0019$ in OS).

Conclusions: Our data indicated that PD-L1 expression was related to higher levels of TILs, and PD-L1-positive tumors with low-TILs were associated with poor prognosis in patients with TNBCs. It is proposed that these biomarkers may be of use for predicting their prognosis and essential in the subclassification of TNBCs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-06

Title: Effect of serum biomarkers (activin A, CAIX, HER2, TIMP-1, and uPA) on outcome in HER2+ metastatic breast cancer patients treated in first line with lapatinib or trastuzumab combined with taxane: CCTG MA.31

Kang A, Hupp M, Ho D, Huang J, Leitzel K, Ali S, Shepherd L, Parulekar WR R, Ellis CE E, Rocco CJ J, Zhu L, Virk S, Nomikos D, Aparicio S, Gelmon KA A, Truica C, Al-Marrawi Y, Rizvi S, Vasekar M, Nagabhairu V, Polimera H, Marks E, Richardson A, Ali AS S, Krecko L, Carney WP P, Downs S, Chen BE E and Lipton A. Penn State Hershey Medical Center, Penn State Hershey Cancer Institute, Hershey, PA; Queen's University, Canadian Cancer Trials Group; GlaxoSmithSkline; British Columbia Cancer Agency; Pinnacle Health System; Walt Carney Biomarkers Consulting, LLC and R & D Systems, Inc..

Body: Background: In MA.31, the lapatinib-taxane combination led to shorter PFS than trastuzumab-taxane in HER2+ metastatic breast cancer. We investigated the prognostic and predictive effects of pretreatment serum biomarkers.

Methods: MA.31 accrued 652 patients; 537 (82%) were centrally-confirmed HER2+. Biomarkers were categorized for univariate and multivariate predictive investigations with a median cut-point, ULN cut-points (15 ng/ml- HER2; 506 pg/ml- CAIX; 454 pg/ml- TIMP-1; 1940 pg/ml- uPA; 600 pg/ml- activin A), and custom cut-points (30 and 100 ng/ml for HER2). Stratified step-wise forward Cox multivariate analysis used continuous and categorical biomarkers for PFS in the ITT and central HER2+ populations; central HER2+ biomarker results are shown.

Results: Serum was banked for 472 (72%) of 652 patients. Higher serum activin A (>median; >ULN; $p<0.0001$); higher CAIX (>median; \geq ULN; $p=0.02$; $p=0.001$); higher HER2 (>median; ≥ 15 ; ≥ 30 ; or ≥ 100 ng/ml; $p=0.05-0.002$) and higher TIMP-1 (>median; \geq ULN; $p=0.001$; $p=0.02$) had shorter univariate PFS. In multivariate analysis for PFS: higher continuous activin A (HR=6.75 with Box-Cox transformation, $P<0.0001$) was associated with significantly shorter PFS, along with treatment arm, prior adjuvant anthracyclines, and higher central EGFR status. In multivariate analysis for OS: higher continuous activin A (HR=85.9, with Box-Cox transformation, $P<0.0001$) was associated with significantly shorter OS, along with treatment arm and higher central EGFR status. The interaction terms of serum biomarkers with treatment were not significant. Elevated serum activin A was also significant at the median cutpoint for PFS (HR 1.79, $p=0.0002$) and OS (HR 2.39, $p=0.006$) in multivariate analysis.

Conclusions: Higher serum activin A was a significant independent prognostic biomarker of shorter progression-free and overall survival. No serum biomarker was predictive of differential response to lapatinib vs. trastuzumab. Evaluation of activin A and CAIX-targeted therapy in addition to HER2-targeted therapy may be warranted in patients with elevated serum levels of these biomarkers.

*AK, MH, DH, & JH contributed equally Grant: PA Breast Cancer Coalition.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-07

Title: ESR1 amplification and 5'-3' exon imbalance in metastatic breast cancer

Oesterreich S, Basudan A, Preideigkeit N, Hartmaier RJ J, Bahreini A, Gyanchandani R, Leone JP P, Lucas PC C, Hamilton RL L, Brufsky AM M and Lee AV V. University Of Pittsburgh Cancer Institute, Pittsburgh, PA; Foundation Medicine, Cambridge, MA and University of Iowa Carver College of Medicine, Iowa City, IA.

Body: BACKGROUND: Growing evidence indicates that base pair mutations in *ESR1* are relatively uncommon in newly diagnosed, treatment-naive breast cancer, but frequently acquired in hormone-resistant metastatic breast cancer (MBC). We and others have recently identified *ESR1* gene fusion and amplification in MBC, with the *ESR1* fusions generally encompassing AF1 and the DNA binding domain. The genomic break required for gene fusions often results in an imbalance in the DNA copy number of exons around the break. We examined *ESR1* amplification and 5' and 3' exon copy number imbalance in MBC.

MATERIALS and METHODS: We designed NanoString DNA hybridization probes against coding and non-coding exons (n=9) in *ESR1* and 15 reference probes. We analyzed 128 samples consisting of 61 ER-positive and 44 ER-negative metastases, and 23 primary breast cancers. DNA copy number (CN) was determined using nSolver, with >2.7CN as copy number gain, and >10 as CN amplification. *ESR1* CN was calculated by averaging the DNA copy number obtained from all coding exons. The 5'-3' copy number ratio was the average copy number of the 5' exons (3-6) divided by the 3' exons (7-10).

RESULTS: 8 (13%) ER positive metastatic breast cancers showed *ESR1* amplification with 5 (8%) having >2.7CN, and 3 (5%) with >10CN. In contrast, in ER-negative metastases, we did not detect any samples with amplification >10CN, and a gain (>2.7 CN) in one case. Similarly, in ER+ primary cancers we did not detect any samples with >10 CN amplifications and 2 samples with CN gain (>2.7 CN). *ESR1* showed 5'-3' CN imbalance in 1 primary (5%) and in 5 metastatic (5%) breast cancers. We are currently confirming and expanding these data in a larger dataset.

CONCLUSIONS: In addition to *ESR1* mutations, *ESR1* CN amplifications and 5'-3' imbalance are represent frequent occurrences in endocrine resistant breast cancer. Future studies are aimed at understanding whether the observed exon imbalances are associated with generation of fusion proteins, and whether and how *ESR1* amplifications cause changes in endocrine treatment response.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-08

Title: The complete spectrum of *ESR1* mutations from 7590 breast cancer tumor samples

Spoerke JM M, Schleifman E, Clark TA A, Young G, Nahas M, Kennedy M, Young L, Chmielecki J, Otto GA A, Lipson D, Wilson TR R, Gendreau S and Lackner MR R. Genentech, Inc, South San Francisco, CA and Foundation Medicine, Inc, Cambridge, MA.

Body: Background: Approximately 70% of newly diagnosed breast cancers express estrogen receptor alpha (ER α), and are treated with agents that block ER signaling. Acquired mutations in *ESR1*, the gene that encodes ER α , have been associated with resistance to aromatase inhibitor therapy in patients with ER positive metastatic breast cancer (ER+ mBC). The most frequently occurring *ESR1* mutations are clustered between amino acids 536 to 538 within the ligand binding domain (LBD), although limited data exists characterizing the full mutation profile in a large number of breast cancer samples.

Methods: We surveyed the Foundation Medicine dataset of 7590 primary and metastatic breast cancer tumor samples for *ESR1* short variants and copy number alterations. Hormone receptor status was unavailable, therefore two assumptions were made to provide an estimate of prevalence in the ER+ HER2- population: 70% of the tumor samples are from ER+ HER2- patients, and all *ESR1* mutations from non-*HER2* amplified metastatic sites are from ER+ HER2- patients. In a separate cohort of 48 ER+ mBC patients, circulating tumor DNA (ctDNA) was analyzed for *ESR1* mutations using the BEAMing method by Sysmex and with Foundation Medicine's sequencing assay, FoundationACT (Assay for Circulating Tumor DNA).

Results: The prevalence of mutations in ER+ HER2- breast cancer was estimated to be 22% in samples from metastatic sites but less than 3% in samples from primary sites. *ESR1* amplification was rare in samples from both primary and metastatic disease sites at 1.3% and 2.0% respectively. A total of 153 unique short variants of known and unknown status were identified. In addition to hotspot mutations at 537 and 538, previously undescribed rare mutations were identified throughout the entire length of the LBD, although 10 alterations at amino acids 380, 463, 536, 537, and 538 account for 86% of all *ESR1* mutations in the ER+ HER2- metastatic sites. We also characterized the overlap of *ESR1* alterations with commonly altered and clinically relevant genes in breast cancer, including *PIK3CA* mutations and *HER2* amplification, and we report here a landscape of co-occurring alterations. In the cohort of patient samples where ctDNA was analyzed, BEAMing and FoundationAct assays both detected *ESR1* mutations in 19 out of 48 samples, and overall concordance of mutation status (wild-type vs mutant) was 100%. A total of 51 individual mutations were detected with the BEAMing assay, 42 of which were detected with the FoundationACT assay. Seven mutations that were undetected by FoundationACT had mutant allele frequencies less than 0.1%. Ten *ESR1* mutations were detected only by FoundationACT, 9 of which are not covered with the BEAMing assay. Alterations in *PIK3CA*, *CDH1*, *TP53*, *ERBB2*, and other breast cancer relevant genes were also detected with FoundationACT.

Conclusions: Understanding the mutational landscape of *ESR1* and co-occurring alterations is important for diagnostic development in conjunction with the clinical development of novel anti-endocrine therapies. Our data demonstrate a large spectrum of mutations in the LBD in addition to known hotspot mutations. In addition, the FoundationACT assay offers a robust NGS-based method to screen for mutations in ctDNA that is highly concordant with digital PCR methods.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-09

Title: Biomarker analysis from the neoadjuvant KRISTINE study in HER2-positive early breast cancer (EBC)

de Haas SL L, Hurvitz SA A, Martin M, Kiermaier A, Lewis Phillips G, Xu J, Helms H-J, Slamon D and Press MF F. F. Hoffmann-La Roche Ltd.; David Geffen School of Medicine, University of California Los Angeles; Hospital Gregorio Marañón, Universidad Complutense; Genentech, Inc. and Keck School of Medicine, USC/Norris Comprehensive Cancer Center.

Body: Introduction KRISTINE (NCT02131064) is an open-label, phase 3 study of neoadjuvant trastuzumab emtansine + pertuzumab (T-DM1+P) vs docetaxel + carboplatin + trastuzumab + pertuzumab (TCH+P) in pts with HER2+ EBC (all pts, Table). In exploratory analyses we assessed whether HER2 expression and gene amplification levels and *PIK3CA* mutation were associated with pathologic complete response (pCR) in KRISTINE.

Methods Treatment-naïve pts with stage II–IIIC HER2+ EBC were randomized to receive 6 cycles of T-DM1+P or TCH+P and assessed for the primary endpoint, pCR (ypT0/is, ypN0). HER2 status was confirmed centrally as immunohistochemistry (IHC) status of IHC3+ and/or HER2/CEP17 gene ratio ≥ 2 by in situ hybridization (ISH) before study entry. Baseline HER2 mRNA, *PIK3CA* mutation, and hormone receptor (HR) status were evaluated. Rates of pCR were compared across biomarker subgroups, including: HER2 mRNA, HER2 IHC staining percentage, HER2 gene ratio, and *PIK3CA* mutation. Incidence of overlap between biomarkers was assessed. All analyses were descriptive.

Results KRISTINE randomized 444 pts (data cutoff, Dec 3, 2015; TCH+P=221; T-DM1+P=223). The biomarker population was representative of the ITT population. Biomarker values and distribution were balanced across treatment arms. Lower pCR rates were seen in both treatment arms across biomarker subgroups with lower vs higher HER2 expression levels (Table). *PIK3CA* mutation was associated with numerically lower pCR with T-DM1+P but not TCH+P. Evaluation of 15 pts who progressed during T-DM1+P neoadjuvant therapy suggests that nonresponse to therapy in this arm may be associated with low HER2 levels (mRNA, IHC, and ISH).

PIK3CA mutation rate was higher in tumors with \leq median HER2 mRNA (38%) vs $>$ median (16%), and in focal (53%) and variable (41%) HER2 expression vs homogeneous (24%). A numerically higher proportion of HR positivity was seen in the HER2 IHC2+ (81%) subgroup vs IHC3+ (55%), and in the focal (83%) and variable (62%) HER2 subgroups vs homogeneous (53%).

Table

Biomarker	TCH+P		T-DM1+P		Difference in pCR rates (%)	95% CI
	n	pCR (%)	n	pCR (%)		
All pts	221	55.7	223	44.4	-11.3	-20.5, -2.0
HER2						
IHC3+	194	60.8	195	49.7	-11.1	-20.9, -1.3
IHC2+	25	20.0	28	7.1	-12.9	-31.2, 5.5
HER2 IHC 2+/3+ fraction*						
Focal (<30%)	15	33.3	16	0	-33.3	-57.2, -9.5
Variable (30–79%)	27	29.6	27	22.2	-7.4	-30.7, 15.9
Homogeneous (\geq 80%)	179	61.5	180	51.7	-9.8	-20.0, 0.4
HER2 mRNA expression						
\leq median	106	49.1	109	37.6	-11.4	-24.6, 1.7
$>$ median	107	63.6	108	51.9	-11.7	-24.8, 1.4
HER2/CEP17 gene ratio						
≥ 2 to < 4	45	33.3	52	19.2	-14.1	-31.6, 3.4
≥ 4	166	62.0	158	53.2	-8.9	-19.6, 1.8
PIK3CA mutation						

Mutated	53	54.7	61	31.1	-23.6	-41.3, -5.8
Nonmutated	160	56.3	151	51.0	-5.3	-16.3, 5.8

*Categorization based on sum of IHC2+ and IHC3+ staining percentage.

Conclusions None of the evaluated biomarker subgroups showed superior pCR rates for T-DM1+P vs TCH+P. Lower HER2 levels based on mRNA and protein expression and gene amplification were associated with numerically lower pCR in both treatment arms. *PIK3CA* mutation results differ from prior observations. Unfavorable biomarkers for pCR, like low HER2, *PIK3CA* mutation, and HR positivity, showed a tendency for co-expression, which should be considered when planning future trials in HER2+ BC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-10

Title: DNA methylation signature predicting bevacizumab efficacy in metastatic breast cancer

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Body: Background: Biomarkers predicting response to bevacizumab containing therapy in breast cancer are still missing. Since epigenetic modifications can contribute to an aberrant regulation of angiogenesis and to treatment resistance, we investigated the influence of DNA methylation on bevacizumab efficacy.

Patients and methods: A genome-wide methylation profiling using the *Illumina Infinium HumanMethylation450 BeadChip* was performed in archival FFPE specimen of 36 patients with HER2-negative metastatic breast cancer treated with chemotherapy in combination with bevacizumab as first-line therapy (*learning set*). Based on objective response and progression-free survival (PFS) and considering ER expression, patients were divided in responders (R) and non-responders (NR). Differentially methylated gene loci (methylation variable position = MVP) and differentially methylated regions (DMR) between R and NR were identified. Only significant sites with a strong change in methylation levels ($\Delta\beta > 0.15$ or $\Delta\beta < -0.15$), an area under the curve of at least 0.85 by logistic regression analysis, and/or sites in proximity to genes functionally involved in angiogenesis and carcinogenesis were selected and further validated. Validation was performed in 81 bevacizumab treated breast cancer patients (*validation set*) and in 15 patients treated with chemotherapy only (*control set*) using targeted bisulfite sequencing. Methylated gene loci were considered predictive if there was a significant association with outcome (PFS) in the *validation set* but not in the *control set* using Spearman rank correlation, Cox regression, and logrank test.

Results: In the *learning set* 435 MVPs ($p < 0.001$) and 144 DMRs (adjusted combined $p < 0.0001$ with at least 3 significant MVPs $p < 0.05$ in the same region) showed significantly different methylation between R and NR. In R 152 sites were hypermethylated with a median change of methylation ($\Delta\beta$) of 0.13; 283 sites were hypomethylated with a median $\Delta\beta$ of -0.09. A methylation signature of 48 genes was specified allowing a good separation between responders and non-responders (odds ratio=101, $p < 0.0001$; data presented at the *38th SABCS 2015, P3-07-43*). At least one methylated cytosine in close proximity to 24 of these genes showed a significant association with PFS in the *validation set* but was not (or in the other direction) in the *control set*. Based on these data the methylation signature predicting long lasting response to bevacizumab could be reduced to 24 genes including several genes involved in angiogenesis and carcinogenesis, respectively (FLT1 also known as VEGFR-1, MLH1, GNAS, APC, DKK3, WNT2B and COL4A1).

Conclusion: A 24-gene methylation signature can reproducibly discriminate between responders and non-responders to a bevacizumab-based therapy in breast cancer and could help to identify patients deriving greater benefit from anti-VEGF agents.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-11

Title: Serum levels of RANKL are increased in primary breast cancer patients in the presence of disseminated tumor cells in the bone marrow

Kasimir-Bauer S, Bittner A-K, Goebel A, Hoffmann O, Browne AJ J, Rauner M, Hofbauer LC C, Wimberger P, Kimmig R and Rachner TD D. University Hospital Essen, Essen, Germany; University Hospital Dresden, Dresden, Germany and University Hospital Dresden, Dresden, Germany.

Body: Background: Receptor activator of nuclear factor kappa-B ligand (RANKL) is an essential protein for osteoclast regulation that is associated with benign and malignant bone disease. The activity of RANKL is controlled by its soluble decoy receptor osteoprotegerin (OPG). There is increasing evidence that RANKL may also directly affect breast cancer progression and metastasis to the bone. This study was aimed to assess the levels of RANKL and OPG in 509 newly diagnosed breast cancer patients with regard to the presence of disseminated tumor cells (DTCs) in the bone marrow (BM), circulating tumor cells (CTCs) in blood and clinical parameters.

Patients and Methods: 509 patients with first diagnosis of breast cancer between Aug 2006 and Dec 2009 were included in our study. Blood and BM sampling was performed before surgery in an adjuvant setting. Blood was collected from each patient and sRANKL as well as OPG in the serum were measured by ELISA (Immunodiagnostic, Vienna, Austria). Two BM aspirates were analyzed for DTCs by immunocytochemistry using the pan-cytokeratin antibody A45-B/B3. In a subgroup of 364 patients, 2 x 5 ml blood was studied for CTCs using the AdnaTest BreastCancer (QIAGEN, Hannover GmbH, Germany) for the detection of EpCAM, MUC-1, HER-2, and beta-Actin transcripts.

Results: Mean serum values for RANKL and OPG were 0.23 ± 0.20 pmol/l and 4.24 ± 1.70 pmol/l, respectively. RANKL levels were significantly lower in women above 60 years of age (0.19 pmol/l vs 0.26 pmol/l; $p < 0.0001$). This finding was reflected by higher RANKL serum levels and RANKL/OPG ratios in premenopausal patients compared to peri- ($p < 0.05$) and postmenopausal patients ($p < 0.001$), respectively. RANKL/OPG ratios were also higher in patients with lymph node involvement (N1-N3, $p = 0.03$). All other clinical parameters did not influence RANKL or OPG levels. DTCs were detected in 213/509 (42%) patients and CTCs in 81/364 (22%) patients, respectively. However, while RANKL levels were unchanged in patients with detectable CTCs, they significantly increased by 33% ($p < 0.0001$) in patients with DTCs. There was no difference in OPG levels, resulting in an increased RANKL to OPG ratio in patients with DTCs in the BM (0.087 vs. 0.060 ; $p < 0.0001$).

Conclusion: In conclusion, we show that RANKL serum levels and RANKL/OPG ratios are increased in patients with detectable DTCs in the BM, prior to the establishment of detectable bone metastases. This finding warrants further investigation as it may provide a rational for novel diagnostic or therapeutic approaches.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-12

Title: A physical activity and dietary counseling intervention in breast cancer survivors and changes in known and novel prognostic biomarkers

Peterson LL L, Ford ME E, Gregoski MJ J, Knight KD D, Hilton EJ J, Magwood G and Turner DP P. Washington University, St. Louis, MO and Medical University of South Carolina, Charleston, SC.

Body: High rates of overweight/obesity are commonly seen in breast cancer (BC) survivors. Observational data show an association between post-treatment increased weight and lack of physical activity (PA) and risk of BC recurrence and death. Increases in prognostic inflammatory associated biomarkers (BM) such as interleukin-6 (IL6) and C-reactive protein (CRP), and their downstream effects, are linked to overweight/obesity and provide a potential mechanistic explanation for this increase in recurrence risk, but studies are mixed regarding the effect of lifestyle interventions on these BM. In addition, better BM may exist. The Getting on Board with an Active Lifestyle (GOAL) study tested the feasibility of a PA and dietary counseling (DC) intervention in BC survivors and included known BM (IL6, CRP) as well as a novel BM (advanced glycation end-products [AGEs]). AGEs are reactive metabolites produced by an uncontrolled reaction between sugars and proteins and were selected because they are seen in chronic diseases including: diabetes, neuro-degenerative disorders, stroke, heart disease and more recently, BC. Further, dietary AGEs are consumed in high fat and highly processed foods that contribute to overweight/obesity.

Methods: Ten overweight/obese women (BMI \geq 25) within 36 months of BC diagnosis (stage I-III) participated in a 12-week supervised PA and DC intervention consisting of two supervised PA sessions per week and weekly DC sessions. Body mass index (BMI), resting heart rate (HR) and blood pressure (BP) and blood samples were collected at baseline, week 4, 8, 12, 24, 36, 52. IL-6, CRP and AGEs were assessed in serum using commercially available 96-well format ELISAs. Data through week 12 is presented here.

Results: Ten participants (four African American) completed the 12-week intervention. The age range of participants was 50-68 years (mean 56 years). The average number of daily active minutes increased significantly between baseline (45) and week 11 (71). There was a drop off at week 12 due to right-censoring of the data. Dietary AGE intake decreased in 8 of 10 participants from baseline to week 12 (average reduction 53%). Significant reductions in mean serum AGEs were seen (baseline=53 ug/ml, week 12=38ug/ml, $p<0.001$). No correlating reductions in CRP or IL6 were found. Correlations were seen between AGE levels and AGE intake ($r=0.24$ at week 12). There were no significant correlations between AGE levels and IL6 or CRP. Decreases in BMI (average change -0.54 kg/m²), resting HR and BP corroborated with AGE reductions.

Conclusions: The GOAL intervention has the potential to improve PA and dietary AGE intake among overweight/obese BC patients. Participants improved weight, resting HR, BP, and number of daily active minutes; which are important metrics for overall health. There were no changes in IL6 and CRP, but reductions in AGEs correlated with reductions in dietary AGE levels, indicating that serum AGEs may be reduced through diet and PA. Serum AGEs may represent a better BM than IL6 and CRP in BC survivors. Further investigation of AGEs in BC survivors is warranted.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-13

Title: Association of pre-chemotherapy peripheral blood pro-inflammatory (IL-6, CRP) and coagulation (D-dimer) markers with chemotherapy toxicity in women with breast cancer

Yuan Y, Vora N, Sun C, Li D, Mortimer J, Luu T-h, Somlo G, Waisman J, Chao J, Katheria V, Synold T, Tran V, Mi S, Levi A, Yost S, Arsenyan A, Choi J, Zavala L and Hurria A. City of Hope, Duarte, CA.

Body: Background: Pro-inflammatory and coagulation factors serve as biomarkers of aging and functional reserve. Chemotherapy (chemo) decreases the risk of relapse and mortality from breast cancer (BC); however, it comes with the risk of toxicity. The utility of these markers as biological risk factors for chemotherapy toxicity in patients with BC is unknown. This study was performed to determine if pre-chemo IL-6, CRP and D-dimer were associated with chemotherapy toxicity in women with breast cancer receiving adjuvant or neoadjuvant chemo.

Methods: This study enrolled women across the aging spectrum with Stage I-III BC. Prior to (neo)adjuvant chemo initiation, peripheral blood was collected for IL-6, CRP, and D-dimer. (Neo)adjuvant chemo regimens were prescribed at the MD's discretion. Grade 3 or above toxicities defined by National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.0, were captured. Univariate and multivariate analyses were performed to describe the association of these biomarkers with chemo toxicity controlling for relevant tumor and host factors (stage, receptor status, age and co-morbidities).

Results: 159 patients (mean age of 58.4, range 30-81) with stage I-III BC (Stage I [n=34; 21.3%], Stage II [n=88; 55.3%], and Stage III [n=37; 23.3%]) were enrolled. 89% and 11% received adjuvant and neoadjuvant chemotherapy respectively. Chemo regimens include: doxorubicin+cyclophosphamide/paclitaxel (37%), docetaxel/cyclophosphamide (35%), doxorubicin+cyclophosphamide/paclitaxel/trastuzumab (7%), docetaxel/carboplatin/trastuzumab (7%), sequential doxorubicin/paclitaxel/cyclophosphamide (5) and other regimens (9%). At least one grade 3 to 5 toxicity occurred in 70 (44%) patients (93% grade 3, 6% grade 4, and 1% grade 5). Grade 3 to 5 hematological (heme) and non-heme toxicity occurred in 23% and 39%, respectively. The most common grade 3 to 4 heme toxicities were anemia (38%), leucopenia (29%), and neutropenia (24%). One patient developed grade 5 toxicity (pneumonitis). The most common grade 3-4 non-heme toxicities were electrolyte abnormalities (12%), neuropathy (10%), mucositis (8%), infection (8%) and fatigue (8%). Univariate analysis revealed an association of increased pre-chemo D-dimer and grade ≥ 3 toxicity ($p=0.02$) (Table 1). Among the clinical factors, increased age and number of co-morbidities was associated with grade ≥ 3 toxicities ($p<0.01$ respectively). After controlling for age and number of comorbidities the association between elevated D-dimer and chemo toxicities remain significant (OR 2.1 [95%CI 1.1-3.9]).

Conclusions: Grade 3-5 toxicities are common in women with breast cancer undergoing (neo)adjuvant chemo. A biomarker of aging, D-dimer, is associated with increased risk of chemo toxicity.

Table 1 Association of peripheral blood biomarkers of aging and Grade 3-5 chemo toxicities

	Grade ≥ 3 Toxicity (N=89)	Grade < 3 Toxicity (N=70)	P-Value
IL-6 (pg/ml) Median (Range)	1.7(0 -42.1)	1.9 (0-19.6)	0.57
D-dimer ($\mu\text{g/ml}$) Median (Range)	0.8(0.1-3.3)	0.5 (0.1-2.6)	0.02
CRP ($\mu\text{g/ml}$) Median (Range)	2.6(0.1-48.4)	3.0 (0.2-44.3)	0.57

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-14

Title: New method to measure functional HER2-driven signaling activity in primary tumor cells identifies HER2-negative breast cancers with abnormal HER2 signaling activity: New group of patients may benefit from anti-HER2 therapy

Laing L, Burns D, MacNeil I, Rich B, Huang Y, Myhre S, Soltani S and Sullivan B. Celcuity, Minneapolis, MN.

Body: Background: Clinical trials have indicated a weak correlation between HER2 expression levels and HER2 targeted therapy benefit. Other biological factors, such as HER2 signaling activity, may be important to measure, in addition to expression and amplification of HER2, when identifying patients eligible for HER2 therapies. To measure the HER2-driven signaling activity of a patient's tumor cells, a new assay using an impedance biosensor, the CELx HER2 Signaling Profile (CELx HSP) Test, was developed. This study set out to provide an initial assessment of the CELx HSP Test, specifically to: 1) quantify HER2-driven signaling activity (HER2_S) in cell lines and primary epithelial cells; 2) assess the correlation between HER2 expression levels and HER2 signaling activity; 3) define a preliminary cut-point between normal and abnormal HER2 signaling; and 4) estimate the proportion of HER2- primary breast cancer tumors with abnormal HER2 signaling.

Methods: A training set of de-identified fresh breast tissue specimens was obtained from 50 patients, 34 with HER2- breast cancer (IHC 0 or 1+) and 16 healthy patients. Cell samples were comprised of epithelial cells extracted and cultured from each specimen. Reference human breast cancer cell lines (9 HER2+, 10 HER2-) were also tested, including two cell lines used as controls in IHC HER2 tests. Real time live cell response to specific HER2 agonists (NRG1b or EGF) and with or without an antagonist (pertuzumab, an FDA-approved HER2 dimerization inhibitor) was measured and quantified using an xCELLigence RTCA impedance biosensor (ACEA Biosciences, San Diego, CA). From these responses, the net amount of HER2 participation in HER2 signaling initiated by the HER2 agonists ("HER2_S") was determined. Fluorescence cytometry was used to measure HER2 expression levels of each cell sample.

Results: Of the HER2- breast tumor cell samples tested, 7 of 34 patients (20.5%; 95% CI=10%-37%) had net HER2 signaling activity that was greater than the median HER2_S of the HER2+ cell lines. There was no categorical correlation between HER2 IHC status (+ or -) and HER2 signaling activity (abnormal or normal) (Pearson's Chi-Square = 3.68; Phi Max = -0.78, Contingency Coefficient 0.28). The median HER2_S, or net HER2 signaling activity, was comparable for the HER2- tumor, HER2- cell line, and the healthy patient samples (*Md* = 100, 117, 77, respectively). The median HER2_S in HER2+ cell lines (*Md* = 248) is approximately 2.5-3.0 fold greater than the median of the other groups. A HER2_S above 250 was considered abnormal or test positive, and was defined as the cut-point. The HER2_S for the two IHC HER2 test control cell lines, SKBR3 for HER2+ and MDA-MB231 for HER2-, was 544 and 0.

Conclusions: These findings provide strong evidence that measurement of HER2 signaling activity may provide clinically relevant information, particularly for HER2- breast cancer patients. These results suggest a new group of HER2- breast cancer patients with abnormal HER2-driven signaling may benefit from anti-HER2 therapy. Additional studies are underway to confirm these findings and to analytically validate the CELx HSP test.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-16

Title: Quantitative proteomic analysis of FGFR by mass spectrometry may improve identification of FGFR amplified tumors sensitive to inhibitor therapy

Schwartz S, Tian Y, Fasani R, Diaz Delgado M, Hierro C, Rodon J, Sellappan S, Cecchi F, Hembrough T and Nuciforo P. NantOmics, LLC, Rockville, MD and Vall d'Hebron Institute of Oncology, Barcelona, Spain.

Body: Background: The fibroblast growth factor receptors 1 and 2 (FGFR1 and FGFR2) have been reported as amplified in multiple solid tumors, including lung, breast and gastric cancers. The FGFR family of proteins is an attractive target for therapy due to the importance of FGFR signaling in the pathogenesis of diverse tumor types. However, pan-FGFR inhibitor have shown only modest efficacy in patients with FGFR1 or FGFR2 gene amplification as determined by fluorescence *in situ* hybridization (FISH). Gene copy number alterations may not be the optimal predictive biomarker since the targets of these drugs are FGFR proteins; recent findings suggest that direct measurement of the protein targets of FGFR inhibitor therapies may be necessary to assess their treatment efficacy. We therefore developed quantitative FGFR protein assays using selected reaction monitoring mass spectrometry (SRM-MS). We sought to correlate levels of FGFR1, FGFR2 and pan-FGFR (FGFR1-4) proteins as measured by SRM-MS with FGFR gene amplification status as determined by FISH.

Methods: Formalin-fixed, paraffin-embedded (FFPE) tissue sections from breast (n=18), esophageal (n=1), gastric (n=1), lung (n=2), and endometrial (n=1) tumors were obtained. A board-certified pathologist marked the tumor area for laser microdissection. Tumor cell proteins were extracted using the Liquid Tissue® process and subjected to SRM-MS for analysis of protein expression levels of FGFR1, FGFR2 and FGFR1-4, as well as other targetable proteins including MET, EGFR, and PD-L1. We compared FGFR protein levels with FGFR amplification defined as FGFR to CEP FISH ratio >2.2.

Results: Of 23 tumor samples analyzed, the pan-FGFR1-4 proteomic assay detected FGFR protein in 11 cases (protein expression range: 217.8-3199.5 amol/ug). Ten of these 11 samples (91%) showed FGFR gene amplification. Only a single non-amplified case showed protein expression (235.6 amol/ug). FGFR protein (of 1, 2, and 1-4) was undetectable in 12 samples, of which 5 (42%) harbored FGFR1 amplification. Two of 2 (100%) of FGFR2-amplified cases (average copy number=38) showed high FGFR2 protein expression (3063.0 and 3199.5 amol/ug). Sensitivity of the pan-FGFR1-4 assay was superior to single FGFR assays.

Conclusion: A subset of FGFR-amplified tumors does not express FGFR protein when assessed by highly-sensitive SRM-MS. Patients whose tumors do not express FGFR protein are not likely to respond to FGFR inhibitor therapy, as supported by previous findings in squamous cell lung tumors, in which FGFR1 mRNA and/or protein expression levels greatly outperformed FGFR1 gene copy number in predicting sensitivity to a pan-FGFR inhibitors. An approach combining quantitative proteomics and FISH analyses may accurately identify patients most likely to respond to anti-FGFR protein agents.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-17

Title: A novel fluorescence in situ hybridization assay to detect 9p24.1 amplification in triple negative breast cancer

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Body: Introduction: Previously, we detected amplification of chromosome 9p24.1 encoding *PDL1*, *PDL2*, and *JAK2* (the PDJ amplicon) in up to 25% of triple negative breast cancers (TNBC) using oligonucleotide CGH arrays (aCGH). Amplification was associated with poor outcome and activation of the JAK/STAT pathway. Here, we have developed a novel fluorescence in situ hybridization (FISH) for detection of the JAK2/PDL1 amplification.

Methods: We selected five 9p24.1-amplified and five non-amplified paraffin embedded TNBC tumor samples, defined by aCGH $\log_2\text{ratio} > 2.0$ as amplified for FISH validation. 5' *JAK2* DNA labeled with SpectrumGreen dUTP, 3' *JAK2* DNA labeled in SpectrumOrange dUTP and a commercially available chromosome 9 centromeric probe (Spectrum Aqua) were combined as one probe set. The break-apart (BAP) probe set was applied to individual slides, hybridized, and washed, 50 events/sample were counted. We defined 9p24.1 amplification by FISH as the ratio of average JAK2 score/ average centromere 9 (CEN 9) score > 1.1 . Nonparametric student's *t*-test was used for statistical analysis.

Results: In the amplified subgroup (n=5), JAK2 amplification was detected by FISH with the range from 1.89 to 21.0 (mean, 6.76). To adjust for aneuploidy, the ratio of JAK2 to CEN 9 was measured with the range from 1.02 to 9.21 (mean ratio, 2.9). The sample with highest level of amplification (aCGH $\log_2\text{ratio} = 4$) detected by aCGH also scored highest by FISH (FISH ratio=9.21). One case with JAK2 amplification (aCGH $\log_2\text{ratio} = 3$) was not detected by FISH (ratio, 1.02), but had low tumor cell content. In the non-amplification subgroup (n=5), JAK2 amplification was detected by FISH with the range from 0.98 to 3.54 (mean, 1.8), the ratio of JAK2 to CEN 9 was measured with the range from 0.48 to 1.05 (mean, 0.73). Of note, two tumors with copy number loss by aCGH were confirmed by FISH (ratio 0.48, 0.56). In total, the 9p24.1 amplification was detected in 4 out 5 (80%) amplified samples defined by aCGH and 5 out 5 not amplified. A significant difference in JAK2: CEN 9 ratio ($p=0.03$) was observed between the amplified and non-amplified subgroups but not in JAK2 absolute scores ($p=0.095$).

Conclusion: In this study, we have developed a novel FISH assay for detection of the 9p24.1 amplification in TNBC, encoding *JAK2*, *PD-L1*, and *PD-L2*. The FISH assay correlates with detection of the amplification by aCGH, but is less sensitive, in particular in tissue with lower tumor cell content. We predict that 9p24.1 amplification will be a clinically relevant biomarker in TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-18

Title: A pilot study of the impact of a single dose of zoledronic acid on biomarkers in breast cancer

Cho M, Gong J, Chen S, Yu H, Wong L, Kruper L and Mortimer J. City of Hope, Duarte.

Body: Backgrounds: In randomized trials for early stage breast cancer, patients who received adjuvant zoledronic acid (ZA) had superior disease-free survival and greater tumor shrinkage. Preclinical data suggest that ZA has anti-cancer properties by modulating angiogenesis and tumor immune environment. This is the pilot study to assess changes in tumor biomarkers and immunological function in response to a single dose of ZA in paired tumor specimens (from core biopsy and definitive surgery).

Methods: Postmenopausal women with early stage invasive ductal breast carcinoma who have estrogen receptor and/or progesterone receptor positive tumor for which a lumpectomy or mastectomy was planned prior to systemic therapy were enrolled. A single dose of ZA 4mg IV was given 10-14 days prior to their planned definitive surgery. 14 biomarkers in tumor tissue was assayed; 10 by gene microarray (VEGF, IL1 β , IL-6, MMP-1, MMP-2, MMP-9, TGF, Interferon α and STAT3) and 4 by RT-PCR(P13K, PTEN, IGF-1-R, EGFR. Patient blood samples were drawn at baseline, at 48-72hrs after the single dose of ZA and at the time of surgery and assayed for cytokines and chemokines by using 30-plex Luminex panels.

Results: Eight patients were enrolled in the study (median age 65 and range (57-75); 4 caucations, 2 hispanic and 1 asian; 6 stage I, 1 stage II and 1 stage III). 30-plex luminex assays showed MIG, IP-10 and IL-12 were found to increase in Day 1-3 of ZA therapy from baseline and normalized by Day 10-24. MCP-1, IL2R, MIP-1b and Eotaxin were found to have changes in Day 1-3 from baseline and mix outcome by Day 10-24. EGF, G-CSF, HGF, IFN-alpha and IFN-gamma and RANTES showed no clear pattern among eight patients. Tumor markers and proteomics from pair tumor samples will be presented.

Conclusions: Changes in multiple tumor biomarkers and immunological markers, MIG, IP-10, IL-12, MCP-1, IL2R, MIP-1b, Eotaxin, IFN-alpha and IFN-gamma are seen within 2 weeks of a single dose of ZA.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-19

Title: An alteration of hormonal receptor status throughout tumor progression related to prognosis in breast cancer patients

Lee E-S, Kim J, Yoo T-K, Kim Y, Han J, Kang YJ, Choi J, Rhu J, Lee H-B, Han W, Noh D-Y and Moon H-G. Seoul National University College of Medicine, Seoul, NU, Republic of Korea; SMG - SNU Boramae Medical Center, Seoul, NU, Republic of Korea and Seoul St. Mary's Hospital, The Catholic University of Korea College of Medicine, Seoul, NU, Republic of Korea.

Body: Purpose

We aimed to identify whether hormonal receptors change throughout tumor progression, because this may influence management and influence prognosis in breast cancer patients.

Patients and Methods

From the institution's database, we collected data of 963 patients who developed relapse during their follow-ups. To determine estrogen receptor(ER) and progesterone receptor (PR), we retrospectively reviewed immunohistochemical(IHC) results in both primary and relapsed tumors.

Results

Among a total of 963 patients, 280 and 683 patients experienced locoregional relapse only and distant metastasis irrespective of locoregional relapse, respectively. ER in 650 patients and PR in 590 patients from both primary tumor and relapse were identified, revealing a change in 157 (24.2%) and 154 (26.1%) patients, respectively. In patients with distant metastasis, assessment of ER and PR showed an alteration in 86 and 56 patients, respectively. The overall survival related to the change of ER and PR status in primary tumor and relapse was significantly different (log rank, $P<0.001$ in both ER and PR status). In addition, women with hormone receptors negative primary tumors that changed to hormone receptors positive tumors who received anti-hormonal therapy after relapse showed a statistically significant good overall survival ($p<0.001$) compared with women who had constant ER-negative tumors.(cox regression, hazard ratio 2.32 ; 95% CI, 1.91 to 3.01)

Conclusion

The breast cancer showed alterations of hormone receptor status throughout tumor progression, hat were related to the strategy of treatment and significantly influences survival. Therefore, investigations of hormone receptor at relapse are essential and helpful in breast cancer patient management.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-20

Title: Allele-specific PCR assay for estrogen receptor (ESR1) mutation detection

Litterst CM M, Tran HB, Chien S, Chen X, Wen W and Begovich A. Genomics and Oncology, Roche RMS, Pleasanton, CA; Assay Development, Roche RMS, Pleasanton, CA and Bioinformatics, Roche RMS, Pleasanton, CA.

Body: Seventy percent of breast cancers are estrogen receptor (ER) positive and, while the majority of these patients initially respond to hormone therapy, approximately 20-30% will become therapy refractory. Recent data suggest activating-mutations in the estrogen receptor gene (ESR1) ligand binding domain (LBD), which are acquired during anti-estrogen treatment and rarely found in primary untreated ER+ breast cancer, are associated with resistance. Some researchers propose that ESR1 mutations are a prognostic and predictive marker, but more studies are needed to assess the clinical utility of the ESR1 mutations. However, current ESR1 mutation detection methods, that is, next generation sequencing and digital PCR, are very labor-intensive, lengthy, and expensive and require special training.

To aid research on the diagnostic potential of ESR1 mutations, we developed a prototype allele-specific qPCR assay for the detection of 18 recurrent mutations in the ESR1 LBD. The test is suitable for DNA obtained from FFPE tissue as well cfDNA from plasma. It is sensitive, yet simple, robust and results can be obtained in less than 8 hours. Herein, we demonstrate the analytical performance of our ESR1 mutation detection assay using contrived samples. We are also planning to test clinical FFPET and plasma samples in the future.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-21

Title: High reduction of circulating tumour cells in HER2-negative locally advanced or metastatic breast cancer (LAMBC) patients treated with eribulin as third line chemotherapy (ONSITE study)

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Body: Background Circulating tumour cells (CTC) may be a good biomarker to guide the management of patients with LAMBC (Cristofanilli, NEJM 2004). Detection of CTCs in peripheral blood is a simple procedure. Eribulin monotherapy has shown improved survival in patients with LAMBC that has progressed after 2 or 3 chemotherapy regimens (EMBRACE study). The aims of this study were to evaluate the kinetics of CTCs before and after eribulin treatment, and its correlation with clinical outcomes.

Methods Patients received eribulin (1.23 mg/m^2 on days 1 and 8 of every 21-day cycle) as third-line therapy until progression, unacceptable toxicity or withdrawal. CTCs were measured in 7.5ml of blood at baseline and after the second cycle of treatment. Cell counting was performed using the CellSearch System, Veridex. Cox proportional hazards regression modeling was used to identify independent prognostic factors for survival endpoints.

Results Out of 59 eligible women (mean age 57.7 years), 58 (98.3%) had received previous taxanes and/or anthracyclines. Nearly all (98.3%) had HER2-negative tumors, 72% were positive for estrogens, 21% were triple-negative; 64.4% had liver metastasis and 57.6% bone metastasis. The mean number of administered cycles was 6.9 ± 5.4 . Follow-up was performed in 54 patients, with 18.5% (n=10) partial response, 42.6% (n=23) stable disease, and 38.9% (n=21) progressive disease. Clinical benefit was achieved in 33 patients (61.1%). Median progression-free survival (PFS) was 5.13 months (95% CI 3.23, 8.90) and median overall survival (OS) was 13.6 months (95% CI 11.8, not reached). CTC levels were measured in 50 patients. The mean number of CTCs at baseline was 16.8 (IQR 0-21) and on cycle 2, 5.4 (IQR 0-8.5), $p < 0.001$. In patients with $\text{CTC} \geq 5$ at baseline, 52.4% reduced their levels to $\text{CTC} < 5$ on cycle 2 ($p = 0.043$). No significant differences ($p = 0.066$) were found in PFS when baseline CTC levels were < 5 or ≥ 5 cells/ml. Statistical differences were, however, found in OS ($p = 0.0083$). On cycle 2, significant differences were observed for both PFS ($p = 0.045$) and OS ($p = 0.0129$) with CTC levels < 5 or ≥ 5 cells/ml. No correlation could be found between CTC levels and the objective response at baseline or on cycle 2.

Conclusions Our study suggests there is a significant correlation between levels of CTCs and disease prognosis. Eribulin monotherapy was related to a significant reduction in CTCs.

(EudraCT number 2013-001416-30).

Keywords eribulin, metastatic, breast cancer, circulating tumour cells.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-22

Title: Association of Hedgehog signaling pathway with luminal B sub-type of breast cancer affected patients of Pakistan

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Body: Introduction

Hedgehog pathway dysregulation is observed in different types of cancers including breast. In the present study, expression profiles of hedgehog pathway molecules in breast cancer cohort of Pakistan and their probable association with molecular sub-types were explored.

Methods

The study was preceded with ethical approval and informed consent from respective institutions and participants. During 2013-2015, a total of 150 cancer biopsies along with adjacent normal tissues were prospectively collected immediately after surgery and processed for RNA isolation. Transcriptional profiles of salient members including SHH, DHH, IHH, PTCH-1, SMO and GLI-1 were quantified using qRT-PCR. Cohort was categorized into molecular sub-types following St. Gallen International Expert Consensus System. Association of expression levels of these aforementioned molecules with various clinico-pathological parameters was explored.

Result

Both SHH ($p=0.01$) and DHH ($p<0.001$) showed elevated expression among tumors in comparison to their controls. Similarly, PTCH-1 ($p<0.001$) and GLI-1 ($p=0.002$) were also significantly up regulated in the cohort. Interestingly, strong positive correlations were observed among the pathway molecules (r -value ranging from 0.45 to 0.81) which highlight their interdependence towards tumor progression. A significant correlation of SHH, DHH, PTCH-1 and GLI-1 was observed with advanced tumor sizes, stages, grades and nodal involvement ($p<0.05$). Association of IHH, SMO and GLI-1 over expression with cancer metastasis was also established in the cohort. SHH, PTCH-1 and GLI-1 were significantly linked with laterality, age and menopausal status. Expression of SHH ($p=0.002$) was more related to younger age group (mean age < 45 yrs) patients in comparison to elderly women.

Moreover all hedgehog molecules were strongly related to hormonal receptors (ER and PR) (r -value ranging from 0.51 to 0.86) while over-expression of HER-2 was not associated with any pathway component. Briefly, 53% (79) of the cohort was categorized as Luminal-B, 18% (27) triple negative, 15% (23) Luminal-A and 14% (21) HER-2 for sub-typing of breast cancer patients in the cohort. Expression of SHH was significantly associated with the molecular sub-types ($p=0.02$) and age ($p=0.005$) using Pearson Chi-Square test. Elevated expression of SHH was observed in 60% of the patients in Luminal B sub-type.

Conclusion

Hedgehog pathway plays a crucial role in breast cancer progression and is found to be activated in Luminal B sub-type in this cohort. As Luminal B is a more aggressive type of breast cancer having poor prognosis and early-onset, association of SHH with Luminal-B and younger age patients signify its importance as a biomarker for early diagnosis of young patients. Hence therapeutic interventions for hedgehog pathway can improve the prognosis of patients categorized as Luminal B subtype of breast carcinogenesis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-23

Title: The ELK3 expression is positively associated with interferon signaling molecules in triple-negative breast cancer

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Body: Background

ELK3 is one of the ETS transcription factor family members and functions as either a transcriptional activator or repressor with or without RAS signaling. ELK3 mainly locates in nucleus but can be exported to cytoplasm in the response to stress activated kinases. Previous studies reported that ELK3 show suppressive effect on the VEGF-induced angiogenesis through inhibiting ETS-1, and ELK3 expression is associated with cell migration and invasion in MDA-MB-231 breast cancer cells. However, the mechanism of ELK3 action associated with immune microenvironment is not defined. In this study, we evaluated the nuclear and cytoplasmic expression of ELK in breast cancer cells and investigated its relationship to subtypes of breast cancer and expression of immune-associated molecules.

Methods

We evaluated the nuclear and cytoplasmic expression of ELK3 by immunohistochemistry and the level of stromal tumor infiltrating lymphocytes (TILs) in hematoxyline and eosin sections of the 224 consecutive breast cancer cohort and the 581 primary triple negative breast cancer (TNBC) cohort. The expression of ELK3 was analyzed in each breast cancer subtype in the consecutive breast cancer cohort. The expressions of immune-associated molecules, including human leukocyte antigen (HLA)-ABC, MxA and protein kinase RNA-activated (PKR), were also evaluated by immunohistochemistry in TNBC cohort. The relationships between expression of ELK3 and immune-associated molecules were analyzed.

Results

The high nuclear expression of ELK3 was more frequently observed in hormone receptor (HR)+/human epidermal growth factor receptor (HER2)- and TNBC subtypes than HR-/HER2+ subtype in consecutive breast cancer cohort ($p = 0.010$ and 0.002 , respectively); 38.4% (48/125) of HR+/HER2- breast cancer, 29.4% (5/17) of HR+/HER2+ breast cancer, 9.5% (2/21) of HR-/HER2+ breast cancer, and 47.5% (29/61) of TNBC ($p = 0.017$). In contrast, the cytoplasmic positivity for ELK3 was less frequently observed in TNBC subtype than HR-/HER2+ subtype ($p = 0.008$); 13.6% (17/125) of HR+/HER2- breast cancer, 35.3% (6/17) of HR+/HER2+ breast cancer, 28.6% (6/21) of HR-/HER2+ breast cancer, and 6.6% (4/61) in TNBC.

In TNBC cohort, nuclear and cytoplasmic positivity rate of ELK3 were 9.3% (54/581) and 6.0% (35/546), respectively. High nuclear expression of ELK3 was significantly correlated with higher histologic grade of tumor ($p = 0.025$) and higher expression of HLA-ABC ($p = 0.001$), MxA ($p < 0.001$), and PKR ($p = 0.001$). In contrast, the cytoplasmic positivity for ELK3 was significantly associated with lower expression of HLA-ABC ($p = 0.031$) and MX1 ($p = 0.040$), and lower level of TILs ($p = 0.004$).

Conclusion

The nuclear and cytoplasmic expression of ELK3 is various in each subtype of breast cancer and associated with immune-associated molecules and lymphoid infiltration in TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-24

Title: miR-19b-3p and miR-4687-5p as novel circulating miRNAs as potential prognostic biomarkers in breast cancer

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Body: Background

Circulating microRNAs (miRNAs) as regulators of gene expression have recently been promising suitable potential biomarkers due to their stability and ease of detection in blood. In this study, we aimed to determine the plasma expression levels of 372 different miRNAs in patients with invasive ductal breast cancer (IDC).

Methods

The expression levels of 372 circulating miRNAs in plasma samples of 20 patients with operable stage 1-III IDC and 10 healthy controls were determined using RT-PCR arrays. Mean ages of patients and healthy controls were 45.9+/-8.8) and 45.4+/- 5) respectively. Of 20 breast tumors, 12 were luminal breast cancer, whereas 8 were non-luminal as pure HER2-neu or triple negative breast cancer. RNA was isolated using miRNeasy Serum/Plasma Kit (Qiagen, Hilden, Germany, Cat. No: 217004). cDNA synthesis was performed according to manufacturer's instructions with miScript II RT Kit (Qiagen, Hilden, Germany, Cat. No: 218161). Serum/Plasma 384 HC PCR arrays with miScript SYBR Green PCR Kit (Qiagen, Hilden, Germany, Cat. No:218076) were used RT-PCR analysis. These assays included 372 miRNAs in addition to housekeeping genes and reaction controls. All reactions were performed in triplicates. Ct values were analyzed via an online software developed by Sabiosciences. P values were calculated using Student's t-test and p values lower than 0.05 were considered significant.

Results

Among 372 miRNAs, 19 were found to be deregulated in plasma samples of patients with IDC. 8 miRNAs were upregulated, and the other 11 were downregulated.

Upregulated and Downregulated Circulating miRNAs in Patients with Invasive Ductal Carcinoma

Upregulated miRNAs	Fold regulation	p value	Downregulated miRNAs	Fold Regulation	p value
miR-29a-3p	2.024	0.032	miR-19b-1-5p	-2.375	0.048
miR-101-3p	2.400	0.040	miR-4732-5p	-2.091	0.015
miR-542-3p	2.291	0.042	miR-4687-5p	-4.623	0.005
miR-199b-3p	2.019	0.020	miR-3135b	-2.792	0.0005
miR-98-5p	2.483	0.003	miR-4770	-2.415	0.0103
miR-424-5p	3.055	0.034	miR-4301	-2.668	0.007
miR-374c-5p	2.110	0.049	miR-1247-5p	-2.813	0.0003
miR-19b-3p	3.759	0.048	miR-1287-5p	-2.202	0.0007
			miR-197-3p	-4.09	0.023
			miR-126-5p	-2.052	0.029
			miR-671-3p	-2.535	0.014

Table 1

miR-19b-3p was the most upregulated miRNA with a fold change of 3.759 (95%CI:1.83- 5.68, p=.048) while miR-4687-5p was the most downregulated with a fold change of 0.216 (95%CI:0.00001-0.43, p=.005).

Conclusion

Our findings indicate that these 19 deregulated circulating miRNAs might be promising biomarker candidates for detection of IDC. The two most deregulated miRNAs were miR-19b-3p and miR-4687-5p. miR19b-3p belongs to a cluster of miRNAs which has been shown to function as oncogenes resulting in the downregulation of tissue factor expression in breast cancer cells. Further

validation studies are ongoing in order to determine their clinicopathological prognostic value in breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-25

Title: Prostate-specific membrane antigen (PSMA) expression in breast cancer and its metastases

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Body: Background:

Prostate-specific membrane antigen (PSMA) is a type II transmembrane glycoprotein with folate hydrolase and neurocarboxypeptidase activity. PSMA has been shown to be consistently expressed in prostatic carcinoma as well as benign prostatic tissue. In addition, PSMA is selectively expressed in the neovascularization of multiple non-prostatic solid tumours including breast cancers. Inducible PSMA specific expression on angiogenic vasculature suggests that PSMA participates in neovessel growth in developing tumours. Treatment of solid tumours with anti-angiogenic agents has become an established paradigm in cancer therapy. Our study aims at detecting protein expression level of PSMA in breast cancer patients with or without metastases both in the primary tumour and metastases tissues.

Materials and methods:

79 patients with histologically proven breast cancer at the department of Gynecology and Obstetrics at the University of Saarland (Germany) were included. 37 patients showed no sign of lymph node or distant metastases (N0M0). 18 patients showed lymph node metastases (N1M0) and 24 patients were diagnosed with distant metastases occurring on different sites (n=5 liver, n=3 lung, n=5 bone, n=7 brain) (M1). Representative formalin-fixed paraffin-embedded tumour blocks from these specimens were obtained from the department of pathology of University of Saarland. Immunohistochemical staining for PSMA and CD31 was performed on parallel representative tumour sections in each case.

Results:

An immunohistochemical PSMA expression could be detected in tumour cells and in the neovascularization of breast cancer in 36 patients, 16% (n=25) of these patients with primary breast cancer and 45% (n=11) with metastatic disease. The expression is higher in patients with lymph node metastases (40%) and even higher in patients with distant metastases (45%). The distant metastases themselves showed the highest expression of PSMA (67%) compared to the PSMA expression of lymph nodes (30%) or the primary tumor (45%) in patients with metastatic disease. Besides these results we could show that the PSMA expression significantly depends on the tumor stage of the primary tumor. Patients suffering of early stage breast cancer (T1=12%) showed significantly less PSMA expression than patients with T2 primary tumor (29%) (p=0,008). The PSMA expression in T3 and T4 staged primary breast cancer was elevated up to 75%.

Conclusion:

This data suggests that PSMA might be involved in the pathogenicity of breast cancer and its metastasis. In addition, PSMA may be an ideal target for diagnostic purposes as well as targeted therapies against metastatic breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-26

Title: ST8SIA1 is over-expressed in triple negative breast cancer and associated with p53 mutations

Yan Y, Nguyen K, Do K-A, Ueno N, Andreeff M and Battula VL. The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Cancer stem cells constitute a small fraction of cells present in primary tumors, are highly tumorigenic, drug resistant and cause metastases. We discovered Ganglioside GD2 as a breast cancer stem cell (BCSCs) marker in triple negative breast cancer (TNBC). GD2 biosynthesis is tightly regulated by the enzyme ST8SIA1 (GD3 synthase). However, expression of ST8SIA1 and its association with different breast cancer sub-types is not known. Here we hypothesize that ST8SIA1 is up-regulated in TNBC or basal-type breast tumors and associated with stemness in primary breast tumors. First, we examined ST8SIA1 protein expression in breast cancer cell lines (n=12) and found up-regulation of ST8SIA1 in basal compared to luminal-type breast cancer cells. To investigate ST8SIA1's expression in primary tumors, we analyzed RNAseq data from the cancer genome atlas (TCGA) data base which has expression data from 1105 primary and metastatic breast tumors as well as adjacent normal tissues. We found that ST8SIA1 expression varies widely among different breast tumors and that 'basal' type tumors express highest levels of ST8SIA1 compared to all other types of breast cancers including luminal-A or luminal-B or HER2 enriched ($p < 0.01$) tumors. In addition, triple negative breast cancer (TNBC, n=115) express 4.63fold higher ST8SIA1 levels compared to hormone receptors positive tumors including ER⁺ or PR⁺ or HER2⁺ tumors (n=852, $p < 0.001$). Survival analysis by log-rank test indicates that patients with ST8SIA1^{high} tumors survive shorter (median survival 2.6 years) compared to patients with ST8SIA1^{low} tumors (median survival 4.3 years). These data suggests that ST8SIA1 expression is associated with basal-like TNBC tumor types and high ST8SIA1 levels in tumors may shorten survival in TNBC patients.

To investigate the association between ST8SIA1 expression and the most commonly mutated genes in breast cancer, we chose the top 20 most frequently mutated genes in TCGA dataset and examined their correlation with ST8SIA1 mRNA expression. We found that, among the top 20 mutations, p53 has a very strong positive correlation with ST8SIA1 expression ($p < 0.00001$). The expression of ST8SIA1 is >2 fold higher in p53 mutated tumors compared to p53 wild type tumors. The other positively correlated mutation was a nuclear envelop protein called Spectrin Repeat Containing, Nuclear Envelope 1 (SYNE1; $p < 0.05$). Mutations in GATA3 are the most negatively correlated ($p < 0.001$) with ST8SIA1 expression. Interestingly, GATA3 plays a role in epithelial cell differentiation in the mammary gland, supporting the notion that ST8SIA1 is a stem cell-associated gene. In addition, correlation of ST8SIA1 mRNA expression with other genes revealed that FOXA1, the protein which is co-expressed with GATA3 and serves as negative predictor of basal type of breast cancer, is down regulated in ST8SIA1^{high} tumors. In conclusion, ST8SIA1 is associated with basal-type TNBC tumors and has a strong positive correlation with p53 mutations and negative correlation with GATA3 mutations. Knowing that TP53 mutations have a major role in tumorigenesis and drug resistance, these data suggest ST8SIA1 as a potential therapeutic target in TNBC patients with p53 mutations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-27

Title: Characterization of neuroendocrine breast carcinomas for biomarkers of therapeutic options

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Body: Introduction: Neuroendocrine breast carcinoma (NBC) is an extremely rare type of cancer, constituting less than 0.1% of all breast tumors, without specific treatment options. We investigated a biomarker database for frequency of molecular markers that may guide personalized treatment choices for these patients.

Materials and Methods: Molecular profiles of 40 breast carcinomas with neuroendocrine features [ER/PR+ (n=20), HER2+ (n=1) and TNBC (n=14)] were assessed (all female patients, mean age: 60.3 years, range: 39-83 years). Gene expression (Illumina DASL microarray platform), protein expression (IHC), gene amplification (ISH) and next-generation sequencing (NGS; TruSeq Illumina platform) were performed.

Results: 57% of NBCs were positive for hormone receptors (ER/PR), 40% were triple negative TNBC and 3% HER2+ subtypes. Therapeutic biomarkers (IHC) that may guide chemotherapies (and used in other primary sites neuroendocrine tumors) included: high TOP2A (85%) for etoposide or doxorubicin, low TS (57%) for 5-fluorouracil and low ERCC1 (45%) for cisplatin. Additional biomarkers for chemotherapy included: high TOPO1 (60%) for irinotecan, low RRM1 (48%) for gemcitabine and low MGMT (57%) for temozolomide. Biomarkers associated with available targeted therapies included: PTEN loss (39%), positive ALK (33%), cKit (30%), EGFR (29%), AR (26%) and PDGFRA (17%). No gene amplifications were detected in *cMET*, *EGFR*, or *TOP2A*. Targeted sequencing analysis of 47 genes detected variants in *TP53*, *PIK3CA*, *ERBB4* and *APC* genes. Gene expression data (included somatostatin receptor gene family- SSTR1/2/3/4/5) was available for 5 patients, for which 3/5 patients exhibited overexpression of at least one SSTR gene.

Conclusions: Molecular profiling by a multiplatform approach reveals potential personalized therapy options for this very rare breast cancer subtype. With recent success of somatostatin analogs for other neuroendocrine tumors, the overexpression of SSTR gene family in NBC is worthy of further investigation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-28

Title: The 21-gene assay in the decision impact assessment of ER+, HER2- Breast cancer: A French real life prospective study

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Body: BACKGROUND: Tumour gene expression analysis is useful in predicting adjuvant chemotherapy benefit in breast cancer (BC). The 21-gene assay test is used to estimate the risk of recurrence and to predict the benefit of adjuvant chemotherapy (ACT) at an early stage of hormone-receptor-positive (ER+) BC. This study aims to examine the implications of RecurrenceScore (RS) testing within the routine clinical practices in France.

METHODS: We conducted a prospective multicentert study, with voluntary participation, involving women with ER+, HER2-, BC. Performing the assay was left at the discretionof the physicians. The final treatment decision was discussed pre and post assay among tumor board (TB). The therapeutic changes after knowledge of RS were collected. RS and classical BC prognostic markers were investigated in parallel including an estimate of the budget impact of the assay.

RESULTS: 603 patients were included in this analysis (tumor size \leq 20 mm: 70.6%; node-negative patients: 61.2%; SBR 2: 74.9%). After the first TB, ACT was recommended for 529 patients (88.0%) and hormonotherapy (HT) alone for 74 patients (12.0%). The RS was low for 59.7% of the cases, intermediate for 34.0% and high for 6.3%. Based on RS results, second TB changed treatment decision for 70.48% of patients, avoiding CT in 64 % of cases. Only 24% received ACT while 76% received HT alone. The calculated budget impact as a minimal estimate according to the French healthcare payer perspective is estimated at a savings of € 570 per patient, corresponding to a global cost-saving of € 221,730.

CONCLUSION: The results of this "real life" study confirm that the 21-gene assay had a significant impact on therapeutic decision. These findings warrant further consideration for the use of this genomic assay in patients with early stage BC in France.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-29

Title: Heterogeneity of PD-L1 expression in primary tumors and paired lymph node metastases of triple negative breast cancer

Li M, Li A, Zhou S, Xu Y, Xiao Y, Bi R and Yang W. Fudan University Shanghai Cancer Centre, Shanghai, China and Fudan University, Shanghai, China.

Body: Background: Programmed cell death ligand 1 (PD-L1) is a potential predictive biomarker of the response to anti-PD-L1/anti-PD-1 therapy in multiple cancers, including triple negative breast cancer. The purpose of this study was to investigate whether PD-L1 expression is homogenous in primary tumors and synchronous axillary lymph node metastases of triple negative breast cancer.

Patients and Methods: PD-L1 expression was immunohistochemically evaluated in 61 triple negative breast cancer patients' primary tumors and paired lymph node metastases. PD-L1 expression in tumor cells and infiltrating immune cells in the primary tumors and associated lymph node metastases was scored separately and was correlated with patients' clinical parameters and prognoses.

Results: PD-L1 expression exhibited spatial heterogeneity in both the infiltrating lymphocytes and tumor cells of primary tumors and lymph node metastases. The PD-L1 expression levels were significantly higher in the infiltrating lymphocytes and tumor cells of the lymph node metastases than in those of the primary tumors. In addition, its expression was more frequent among the lymph node metastases ($p=0.045$). Furthermore, the disease-free survival and overall survival were similar between the primary tumor negative/ lymph node metastasis positive and primary tumor positive/ lymph node metastasis positive patients, both of which exhibited worse disease-free survival than primary tumor negative/ lymph node metastasis negative patients.

Conclusions: The different expression of PD-L1 between the primary tumors and lymph node metastases suggest that the lymph node metastasis PD-L1 status may be used to indicate whether a node-positive triple negative breast cancer patient is suitable for PD-1/PD-L1-targeted therapy in the future.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-30

Title: Identifying novel glycosylation markers in Her2+ breast cancer using MALDI mass spectrometry

Scott DA A, Angel P, Xu H, Drake R and Yeh E. Medical University of South Carolina, Charleston, SC.

Body: Breast cancer characterized by human epidermal growth factor receptor 2 (HER2) overexpression represents approximately 25-30% of all breast cancer cases. Many patients acquire resistance to current chemotherapies, leading to a more aggressive disease state with severe clinical outcomes. Lapatinib, a first generation tyrosine kinase inhibitor of HER2 and EGFR (epidermal growth factor receptor), is commonly used to treat HER2+ breast cancer. Resistance to lapatinib is steadily increasing among HER2+ patients, highlighting the need for therapy development. Identifying markers that predict treatment response or potential drug targets could enhance treatment efficacy and patient survival. To investigate this, we have used MALDI mass spectrometry to identify N-linked glycans specific to human breast cancer cell lines with known resistance and sensitivity to lapatinib treatment, JIMT-1 (resistant) HER2+ and BT474 (sensitive) HER2+. After different lapatinib dose and time course experiments, N-linked glycans were isolated and comparatively profiled by high resolution MALDI mass spectrometry. Differences in the levels of fucosylation and sialylation of glycans from sensitive and resistant cell lines, before and after treatment, were evaluated. In addition, mouse xenograft tumor tissues derived from the same cell lines treated with and without lapatinib were processed for on-tissue imaging of N-glycans using a MALDI imaging mass spectrometry approach. Tissues from HER2+ human breast tumors were also imaged with the same MALDI imaging approach. Cumulatively, these preliminary studies have identified novel glycosylation patterns associated with lapatinib treatment sensitivity and resistance.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-31

Title: Molecular mechanism of alcohol-associated breast cancer

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Body: Epidemiological studies have indicated that alcohol consumption is an established risk factor for breast cancer. The association of alcohol consumption and breast cancer is more pronounced in ER+ cases than in ER- cases. However, this molecular mechanism remains to be determined. Deregulation of RNA polymerase III (Pol III) transcription enhances cellular tRNAs and 5S rRNA production, increasing translational capacity to promote cell transformation and tumor formation. Our results reveal that alcohol increases Pol III gene transcription in both normal and cancer breast cell lines. The induction of Pol III genes by alcohol in ER+ breast cancer cells is significantly higher than in ER- normal breast cells and ER- breast cancer cells. E2 causes slight increase in Pol III gene transcription. The addition of ethanol to this system produces a marked increase. Alcohol increases ER α expression to enhance the cellular levels of Brf1 protein and mRNA. In addition, ethanol markedly stimulates phosphorylation of JNK1. Inhibition of JNK1 decreases ERE-Luc reporter activity and represses expression of ER α , Brf1 and Pol III genes. Reduction of ER α by its siRNA represses Brf1 and Pol III gene transcription. Ethanol with E2 produces larger and more numerous colonies. Repression of ER α or Brf1 inhibits alcohol-induced cell transformation. More interestingly, human biopsies studies show that Brf1 expression is significantly increased in nuclei of breast cancer cells, compared to tissue adjacent to the carcinoma. Together, these results support the idea that alcohol increases ER α expression through JNK1 pathway to elevate Brf1 expression and Pol III gene transcription, leading to greater phenotypic changes. ER α mediates Pol III gene transcription through Brf1, suggesting that ER α play a critical role in alcohol-induced deregulation of Pol III genes in ER+ breast cancer development.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-32

Title: Vitamin D as a potential biomarker of aggressiveness in non-metastatic breast cancer

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Body: Background: Vitamin D (VD) is known to play a proapoptotic and antiproliferative role in cancer cells. Recent studies have shown that low serum VD may be associated with worse prognosis in cancer patients (pts). However, levels of VD are not routinely measured in daily clinical practice.

Methods: We retrospectively evaluated pts diagnosed with non-metastatic breast cancer between January 2010 and November 2015. Serum 25(OH)-VD and bone density were measured any time after surgery, during either adjuvant treatment or the follow-up. VD insufficiency (VDi) and deficiency (VDd) were defined as serum VD levels of 20-30 ng/ml and <20 ng/ml, respectively. Possible association between VD levels and baseline characteristics as AJCC stage, immunohistochemistry (IHC) subtype, ki67 and differentiation grade were studied. Correlation between synchronous densitometry and VD levels was also evaluated.

Results: 98 pts that met the above described criteria were included in the analysis. Median (M) VD was 20.68 (range, 3 – 65.60). 72.4 % of pts had VD under 30 ng/ml (23.4% had VD insufficiency and 49% had deficiency). AJCC stages II-III were more frequent in patients with low VD levels (Normal: 48%; VDi: 52%; VDd: 65%). VD levels were similar across IHC subtypes (Normal VD levels: Luminal A: 51%; Luminal B: 30%; Triple Negative: 14%; VDi: LA: 52%; LB: 35%; TN: 13%; VDd: LA: 39%; LB: 48%; TN: 13%). Undifferentiated tumors were more frequent among pts with VD levels <30ng/ml (Normal: 27%; VDi: 45%; VDd: 45%). Elevated Ki67 was more commonly found in pts with lower VD (Normal: 40%; VDi: 40%; VDd: 61%). Our results did not show correlation between densitometry and VD levels.

Conclusions: Lower levels of 25(OH)-Vitamin D may be associated with more advanced and aggressive non-metastatic breast cancer. 25(OH)-VD should be evaluated at diagnosis. Although it may be a promising biomarker, further research in this field is required.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-33

Title: Metrics of drug sensitivity based on growth rate inhibition correct for the confounding effects of variable division rates

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Body: Drug sensitivity and resistance are conventionally quantified by IC_{50} or E_{max} values, but these metrics suffer from a fundamental flaw when applied to growing cells: they are highly sensitive to the number of divisions that take place over the course of a response assay. Division rate varies with cell line, experimental conditions, and genetic alterations. The dependency of IC_{50} and E_{max} on division rate creates artefactual correlations between genotype and drug sensitivity while obscuring important biological insights and interfering with biomarker discovery. In this work, we derive alternative drug response metrics that are insensitive to number of divisions occurring during the assay. These are based on estimating growth rate inhibition (GR) in the presence of a drug using endpoint or time-course assays. The latter provides a direct measure of phenomena such as adaptive drug resistance.

Using a simple model of drug response, we first show how GR_{50} and GR_{max} are superior to IC_{50} and E_{max} for assessing the effects of drugs in dividing cells. By expressing an oncogene in a transformed cell line, we illustrate how conventional metrics can lead to artefactual connections between mutations and drug sensitivity. We further validate the superiority of GR_{50} over IC_{50} values by reanalyzing a recently published large dataset of drug sensitivity and showing cases where difference in division rates is the only reason why IC_{50} values correlate with tissue type or genetic alterations. Using GR_{50} values prevents these artificial correlations and restores known connections between drug resistance and genomic markers. Finally, we show how GR_{max} values, which reflect efficacy, quantify differences in the phenotypic response and thus can be used to identify new biomarkers of sensitivity.

Adopting GR metrics requires only modest changes in experimental protocols. GR values and metrics can be evaluated using scripts are available on github (www.github.com/sorgerlab/gr50_tools) or using an interactive website: www.grcalculator.org. We expect GR metrics to improve the use of drugs to identify response biomarkers, study mechanisms of cell signaling and growth, and identify drugs effective on specific patient-derived tumor cells.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-07-34

Title: The prognostic impact of inositol polyphosphate 5-phosphatase PIPP (INPP5J) expression in breast cancer tissue

Kondo N, Kim T-S, Wanifuchi Y, Hato Y, Hisada T, Nishimoto M, Nishikawa S and Toyama T. Nagoya City University Hospital, Nagoya, Aichi, Japan.

Body: Background : Inositol polyphosphate 5-phosphatase PIPP (INPP5J) has been identified as a suppressor of oncogenic PI3K/Akt signaling in breast cancer. INPP5J depletion increases transformation and accelerates oncogene-driven tumor growth in vivo, while paradoxically reducing cell migration, invasion, and metastasis. Therefore, we hypothesized that INPP5J gene expression in human breast cancer tissues would be prognostic in early breast cancer patients over long-term follow-up.

Methods: A total of 478 breast cancer tissue samples collected between 2003 and 2008 was available for analysis. We measured INPP5J mRNA using a TaqMan gene expression assay. PIK3CA mutation status was evaluated using a TaqMan mutation detection assay. We then investigated the correlations of clinicopathological factors and prognosis with levels of INPP5J mRNA and the PIK3CA mutation status.

Results: INPP5J mRNA was expressed at a low level in 30.1% (144/478) and at a medium to high level in the remaining breast cancer samples. Low INPP5J mRNA correlated with larger tumor ($p=0.015$), high grade ($p<0.0001$) and, ER-negativity ($p<0.0001$). PIK3CA mutations were detected in 46% (63/138) of patients analyzed. We found that disease-free survival (DFS) was significantly worse in patients with low levels of INPP5J ($p=0.008$). Although DFS and INPP5J levels tended to be associated in estrogen receptor (ER)-positive patients ($p=0.052$), DFS was significantly worse in patients with wild-type PIK3CA and low INPP5J mRNA expression ($p=0.008$).

Conclusion: We shows that the level of INPP5J mRNA expression is prognostics in breast cancer patients and that its prognostic impact is affected by PIK3CA mutation status.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-08-01

Title: A directed siRNA screen identifies INHBA as a major regulator of tumor aggressiveness in basal HER2 breast cancer

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Body: HER2+ breast cancers can be treated with several HER2-targeted inhibitors, but tumors often acquire or have de novo resistance to the therapies, limiting the long term efficacy of these treatments. To identify genes associated with resistance, we performed drug screens on a panel of HER2 positive breast cancer cell lines using lapatinib, then performed statistical analyses to identify differentially expressed genes. We procured siRNA against the top 20 targets expressed in the resistant subset for subsequent viability screening, both in the presence and absence of lapatinib. This screen identified INHBA, a member of the TGF-B superfamily, as a strong mediator of cell growth and lapatinib response in two resistant HER2+ cell lines. Both these lines were of the basal subtype, which tend to be more resistant in our cell line panel. We tested 8 different HER2+ breast cancer cell lines (4 basal and 4 luminal subtype) with siRNA against INHBA. We observed significant inhibition of growth in 3 out of 4 basal lines, with growth rates of 40-55% of scramble control lines. In contrast, none of the luminal lines showed significant growth inhibition when treated with the INHBA siRNA. Furthermore, treatment with INHBA siRNA sensitized cells to lapatinib, with GI50 (dose required to inhibit growth by 50%) dropping from 10uM to less than 2 uM in JIMT1 and 21-MT1 basal lines. We also tested the effects of knockdown in 3-d matrigel cultures, which recapitulated the 2-d findings with decreased growth and increased sensitivity. For the cell line 21-MT1, growth following INHBA siRNA treatment was 12.6% of scramble control, and lapatinib treatment further reduced this to 7.8%, in contrast to scramble control/lapatinib treated cells, which grew at 97.7% of control rate. We performed RNAseq on 21-MT1 and JIMT1 cells treated with siRNA and looked at both relative changes in expression as well as absolute magnitude changes in expression. There were a large number of mitochondrial genes induced in the INHBA siRNA knockdown cells and loss of glycolytic genes such as LDHA compared to scramble control cells, suggesting a change in metabolic activity. We tested this by treating cells with the oxidative phosphorylation inhibitor oligomycin or the glycolysis inhibitor 2-deoxyglucose (2-DG). We found that JIMT1 cells treated with INHBA siRNA were more sensitive to oligomycin but less sensitive to 2-DG compared to scramble treated controls, suggesting a shift from glycolytic to oxidative phosphorylation metabolism. Since this type of metabolic shift has also been associated with reduced invasive capacity, we performed invasion assays on cells treated with INHBA or scramble-control siRNA. We found that knockdown of INHBA reduced invasion of both 21-MT1 and JIMT1 cells, with invasion rates less than 10% of control cells. Finally, we mined expression data sets to determine associations between INHBA levels and outcome, and found that high levels of expression of INHBA were associated with poor outcome in both HER2+ and the basal subtype of breast tumors, but not in luminal tumors. In conclusion, we identified INHBA as a major regulator of metabolism and aggressiveness in HER2+ basal breast cancer cells that is associated with poor outcome in patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-08-02

Title: Disruption of the estradiol-regulated NTN1-UNC5A dependence receptor signaling axis causes a hybrid basal/luminal molecular phenotype in estrogen receptor-positive breast cancer cells

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Body: Luminal subtype of breast cancers that express the estrogen receptor alpha (ER α) represents approximately two-thirds of all breast cancer cases. ER⁺ tumors tend to have the most favorable prognoses when treated with endocrine therapy. However, a relapse or endocrine therapy resistance is often seen in ER⁺ breast tumors. UNC5A belongs to the dependence receptor family which can mediate two different intracellular signals: cell survival, differentiation or migration when engaged with its ligand (such as Netrin-1; NTN1) or cell death/apoptosis in the absence of the ligand. Here we demonstrate that, depending upon the cell type, UNC5A and NTN1 are estradiol (E2)-inducible genes. Using shRNA or CRISPR knockdown strategies, we show that the disruption of the NTN1-UNC5A signaling axis in ER⁺ (MCF7 and T-47D) cells generates a mixed basal-like/luminal phenotype with stem cell-like characteristics. RNA-seq of UNC5A knockdown cells showed deregulated expression of several E2-target genes in both cell lines. Moreover, knockdown of UNC5A resulted in increased cell proliferation, and elevated expression of the E2-inducible anti-apoptotic, BCL2. Furthermore, the expression of $\Delta Np63$ was enhanced in UNC5A knockdown cells. $\Delta Np63$ is a TP53 family transcription factor that promotes breast epithelial stem cell maintenance and basal-like breast cancer. Accordingly, UNC5A knockdown cells displayed cancer stem cell phenotype as evident from ~3-fold increase in the number of CD44⁺/CD24⁻, CD44⁺/EPCAM⁺ and ITGA6⁺/EPCAM⁺ subpopulation compared with control cells. In addition, the expression of NTN4, a pro-angiogenic and lymphangiogenic factor, was increased upon UNC5A knockdown. In vivo, UNC5A knockdown cells implanted in nude mice were able to form tumors in the mammary fat pad independent of E2 supplementation and were able to colonize and develop into overt metastasis in multiple organs such as lungs, ovaries and adrenal glands. Consequently, analysis of mammary fat pad tumors from animals that received UNC5A knockdown cells revealed an increased expression of PECAM1 (CD31), a marker for endothelial cells used to evaluate tumor angiogenesis. In contrast to UNC5A, knocking down NTN1, decreased the expression of BCL2 and TP63 in both cell lines. Thus, knockdown of UNC5A resulted in deregulated expression of E2-regulated genes, E2-independent and anti-estrogen-resistant growth in vitro, and E2-independent tumor formation in xenograft models. Consistent with results of in vitro studies, analysis of tissue samples from breast cancer patients (n=196) revealed that lower expression of UNC5A is associated with lower overall survival ($P < 0.05$). Thus, loss or mutational inactivation of UNC5A could lead to unrestricted E2:ER α signaling and anti-estrogen resistant growth while simultaneously enabling ER α -positive luminal breast cancer cells to acquire basal-like and cancer stem cell-like features.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-08-03

Title: Dynamics of cancer stem cell surface marker, CD44 and CD44v6 in the antitumor efficacy of PARP inhibitor in combination with PI3K pathway inhibitor in TNBC xenograft model

Lin X, Sun Y, Carlson JH H, Williams C, Krie A, Friedman L, De P, Dey N and Leyland-Jones B. Avera Center for Precision Oncology, Sioux Falls, SD and Genentech Inc., SF, CA.

Body: Introduction: Triple Negative (TN) BC has a limited benefit from conventional therapy and a few options for targeted therapy like PARP inhibitor. We reported that doubling down on the PI3K-mTOR pathway enhances the antitumor efficacy of PARP inhibitor in TNBC (*Neoplasia*, 2014). Tumor cells with distinctive stem cell (CSC) like properties exist in carcinomas including breast and CD44+CSC marker expression correlates with decreased survival (*Fillmore and Kuperwasser*, 2008). In TNBC the intratumoral presence of stem cell population is associated with increased aggressiveness. Among many CSC markers, CD44 is best known to have a prognostic role (*Collina et al.*, 2015) and the PI3K/E2F1 pathway has been identified as a potential signaling link to HA/CD44 activation. **Hypothesis:** We hypothesized that xenografts of TNBC cell lines bearing stem cell property will respond in a characteristic way to GDC0980 in combination with ABT888 plus carboplatin. **Methods:** Athymic mice bearing BRCA-competent TNBC xenograft tumors were used to test the combination of GDC-0980 with ABT888 plus carboplatin. Mechanism-based *in vitro* studies were conducted to understand the mode of action of the drugs. IHC for CD44 and CD44v6 was standardized in positive tumor-controls and identified in (1) BC TMAs, (2) TNBC cell lines and (3) patients from our Avera cohort. Finally, the CD44-expression in tumors of different arms of the xenograft study (MDA-MB231 and MDA-MB468) was evaluated independently by a pathologist who was unaware of the study design. **Results:** CD44 was identified primarily in the membrane and to a lesser extent in the cytosol of tumor cells of BC patients (TMA and Avera cohort), TNBC cell lines and xenograft-tumors. GDC-0980 in combination with ABT888 plus carboplatin blocked the growth of established xenograft tumors by 80-90% with a concomitant decrease in Ki67 IHC-levels. Membrane expression of CD44 inversely correlated to tumor sizes which significantly reduced in response to drug combinations. Mechanistically, GDC-0980 treatment led to DNA damage (increased pgH2AX), gain in PAR and a subsequent sensitization of BRCA-competent TNBC cells to ABT888 plus carboplatin with a time-dependent (1) decrease in proliferation signals (pAKT), PAR/PARP ratios, PAR/pgH2AX ratios, live/dead cell ratios, and clonogenic 3D growth & (2) increase in apoptosis markers (cleaved-caspase3, cleaved-PARP and annexinV positivity). The relationship between expression of CD44 and CD44v6 in the xenograft tumors the following treatment is being worked out which will be presented at the meeting. **Significance:** Our findings demonstrated that PARP inhibitor plus carboplatin in combination with dual PI3K-mTOR inhibition effectively reduced the CD44+CSC population in TNBC xenograft tumors. In a BRCA-competent model, PI3K-pathway inhibition not only enhanced the antitumor activity of ABT888 plus carboplatin by inhibiting proliferation and tumor-induced angiogenesis but also by decreasing CD44+CSC. We present the first mechanism-based study to demonstrate the integral role of PI3K-mTOR pathway and DDR pathway in the control of CSC surface marker in orchestrating antitumor actions of PARP inhibitor in TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-08-04

Title: Preclinical efficacy of dasatinib in combination with PARP inhibitor plus standard cytotoxic agent in triple-negative breast cancer xenograft model

Sun Y, Lin X, Carlson JH H, De P, Dey N, Jepperson T, R & D NCI, Williams C and Leyland-Jones B. Avera Center for Precision Oncology, Sioux Falls, SD and R & D Agreements Regulatory Affairs Branch, CTEP, NCI, Bethesda, MD.

Body: Background: Dasatinib is an orally-active ATP-competitive small molecule kinase inhibitor that potently inhibits Abl kinase, Src family kinases and other kinases (Lombardo et al., 2004). Src, one of the key targets of dasatinib is involved in the regulation of cell proliferation, survival and apoptotic ability of cancer cells (Tryfonopoulos et al., 2011; Pusztai et al., 2014). Dasatinib has shown its anti-proliferative and anti-metastatic effectiveness against triple-negative breast cancer (TNBC) in both preclinical and clinical studies (Finn et al., 2011). Several molecular targets including poly ADP ribose polymerase (PARP) are under clinical investigation for the treatment of TNBC. Recently, PARP inhibitors in combination with chemotherapy have shown promising results in this disease in clinical and preclinical studies (Tutt et al., 2010; Kim et al., 2013; De et al., 2014). Here, we **hypothesize** that dasatinib in combination with PARP inhibitor (ABT888) plus standard cytotoxic agent (carboplatin) will attenuate the growth of both TNBC cell lines and xenograft tumors. **Methodology:**We have used BT-20 (*PIK3CA* mutated, H1047R), HCC70 (*PTEN* null), HCC1937 (*BRCA1* mutated, *PTEN* null), MDA-MB-231 (*KRAS/BRAF* mutated), MDA-MB-468 (*PTEN* null) and SUM149PT (*BRCA1* mutated, *PTEN* null) cells for *in vitro* study. Survival/proliferation, colony formation and apoptosis were examined by using 2D proliferative/growth assay, 3D-ON-TOP assays, and annexinV staining respectively. We next studied the activation status of Src and its downstream signaling. We also have evaluated the effects on tumor growth inhibition of dasatinib/ABT888/carboplatin as a single agent or in combination by using mouse xenograft model. **Results:** We observed that 1) Dasatinib inhibited Src activation in all tested lines, induced dephosphorylation of ERK1/2 and S6 RP; 2) level of Cyclin D1 was decreased by dasatinib treatment; 3) high anti-proliferative activities were observed following the treatment of dasatinib along with ABT888 plus carboplatin in both 2D proliferation assay and 3D-ON TOP colony formation assay; 4) dasatinib in combination with ABT888 plus carboplatin inducing early stage apoptosis was seen by Annexin V staining in all tested cell lines; 5) dasatinib alone or combined with ABT888 or carboplatin or in triple combination inhibited tumor growth in TNBC xenograft models, **the best tumor inhibition result was induced by triple combination** (comparing to no treatment control, the mean tumor volume was decreased ~ 87%). **Conclusion:** Our *in vitro* and *in vivo* studies suggest that dasatinib may enhance the antitumor activity of PARP inhibitor plus standard cytotoxic agent in TNBC. Mechanistic studies of xenograft tumor samples are ongoing, the results of which will be presented in the meeting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-08-05

Title: Genome-wide identification of transcripts regulated by estrogen in MCF-7 cells using BrU-seq

Sun J, Capobianco E, Tsinoremas N and Lippman M. University of Miami, Miami, FL and Institute of Clinical Physiology, Pisa, Italy.

Body: Estrogen promotes estrogen receptor (ER)-positive breast cancer cell growth by modulating transcription through ligand activated ER. The spectrum of early ER target genes has been identified through gene expression microarray and more recently with RNA-seq analyses. These methods, although comprehensive, fall short in identifying many ER-regulated transcripts which are either not present in microarray; too abundant to determine estrogen regulated changes among steady-state pools of RNAs; or have very short half-lives and low abundance such as some non-coding RNAs, including RNAs generated by enhancers bound by ER (eRNAs). Global run-on sequencing (GRO-seq) has been used to identify ER target genes and eRNAs in breast cancer cells. It is technically cumbersome and is performed under non-physiological condition.

We have applied BrU-seq technique to identify global transcripts regulated by estrogen in MCF-7 breast cancer cells. The newly synthesized RNAs in MCF-7 cells treated with or without estrogen for various time points, were labeled with 5'-bromo-uridine (BrU). The labeled RNAs were purified by immunoprecipitation using an anti-BrdU antibody and then subjected to RNA sequencing analysis, which provides greater sensitivity than regular RNA-seq analysis from steady-state total RNA samples. Robust estrogen-regulated transcripts can be detected within 30 min of treatment. Significantly more transcripts (both coding and non-coding RNAs) were identified as estrogen targets using BrU-seq analysis than RNA-seq analysis from total RNA pools. Many of these estrogen-regulated transcripts are located close to ER binding sites identified from ER ChIP-seq analysis, indicating a functional role for these ER binding sites in regulating RNA transcription. For estrogen targets identified through both analyses, the magnitude of expression changes (both up- and down-regulation) is usually greater from BrU-seq than from the regular RNA-seq analysis. The dynamics of RNA synthesis and degradation for some unstable RNAs (such as eRNAs) can be interrogated through our data set. Also, RNA pol II elongation rate can be deduced from some long ER target genes (>100 kb) such as GREB1. In addition to identifying estrogen targets, BrU-seq should be a very useful technique in assessing transcriptional changes triggered by various signal transduction pathways.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-08-06

Title: A positive feedback loop couples CD44s and HAS2 for sustained Akt activation and tumor cell survival

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Body: Breast cancer is the second leading cause of cancer death in women worldwide. Despite recent advances in breast cancer treatment, tumor metastasis and recurrence remain the most significant clinical obstacles, largely due to the resistance of residual breast tumor cells to existing therapies. RNA alternative splicing is a fundamental mechanism for gene regulation and protein diversity. Despite the fact that nearly all human genes are regulated by alternative splicing, very limited studies have addressed the role of alternative splicing in tumorigenesis. Our lab uses the cell adhesion molecule CD44 as a model to investigate the role of alternative splicing in breast cancer metastasis. CD44 undergoes extensive alternative splicing, giving rise to the standard (CD44s) and variant (CD44v) isoforms. Previous work from our lab demonstrated that the CD44s isoform is required for Epithelial to Mesenchymal transition (EMT) and breast tumor metastasis. Mechanistically, we have shown that CD44s, but not CD44v, potentiates Akt signaling, promoting cell survival and chemoresistance in breast cancer cells.

To better understand CD44s-mediated downstream pathways critical for breast tumor metastasis, we analyzed CD44s-dependent gene signature and identified Hyaluronan Synthase 2 (HAS2), an enzyme that produces hyaluronan (HA), as one of the most significantly upregulated genes in response to CD44s expression. Interestingly, HA is the major ligand for CD44, and HA/CD44 interaction promotes Akt activation. These results led us to test a hypothesis that a positive feedback loop, involving CD44s and HAS2, sustains Akt signaling and promotes tumor cell survival. Indeed, our results show that CD44s-mediated Akt activation stimulates HAS2 transcription. This increase in HAS2 transcription is mediated by Akt phosphorylation of FOXO1, a transcription repressor inactivated by phosphorylation. We further show that HAS2 and HA activate Akt signaling in a CD44s-dependent manner. Importantly, disruption of this feed-forward loop inhibits EMT and triggers cell death.

PI3K/Akt signaling is hyperactive in more than 70% of breast tumors. Hence, the proteins involved in this positive feedback loop represent very attractive and promising drug targets for breast cancer therapy. Our work has demonstrated how aberrant expression of alternative spliced isoform in breast cancer, through a positive feedback loop, leads to sustained Akt signaling, enhanced cell survival, and a metastatic phenotype. Disrupting this positive feedback loop may provide a foundation for an innovative approach to treat metastatic breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-08-07

Title: Gain and amplification of RAC1 GTP-ase in BC: Explaining alterations in patients by experiments using TNBC model

Dey N, Carlson JH H, Jepperson T, Willis S, De P and Leyland-Jones B. Avera Center for Precision Oncology, Sioux Falls, SD.

Body: INTRODUCTION: RAC1-GTPase which transduces signals from cell surface integrins, have been implicated in metastasis. We reported that Wnt-beta-catenin pathway (WP) that signals metastasis (*BMC Cancer, 2013*), is one of the salient genetic features of Triple-Negative Breast Cancer (TNBC) (*PlosOne, 2013*). **AIM:** We demonstrated that TNBC cells acquire integrin-directed metastasis-associated (ID-MA) phenotypes following an upregulation of the WP (Oncotarget, *In Press*). Here we examined how WP signals are transduced in the context of ID-MA phenotypes in TNBC. **METHOD:** We documented gain and amplification of *RAC1* gene in Breast Invasive Carcinoma subtypes from cBioPortal. The outcome for RFS was studied in the Hungarian ER-ve BC cohort. Mechanistically, we studied fibronectin-directed (1) migration, (2) matrigel- invasion, (3) RAC1 activation, (4) actin dynamics (confocal microscopy) and (5) podia-parameters using pharmacological agents (sulindac sulfide), genetic tools (beta-catenin siRNA), WP modulators (Wnt-C59, XAV939), RAC1 inhibitors (NSC23766, W56) and WP stimulations (LWnt3ACM, Wnt3A recombinant) in a panel of 6-7 TNBC cell lines, **RESULTS:** The collective percentage of gain and amplification of *RAC1* were (1) 31% of total 1105 breast invasive carcinoma samples, (2) 29% of total 594 ER+ve samples, (3) 39% of total 174 ER-ve samples, (4) 38% of total 120 HER2+ve samples and (4)35% of total 82 TNBC samples ([brca/tcga/pub2015](#); Cell 2015). In invasive ductal BC subtypes, gain and amplification of *RAC1* were (1) 32% of total 201 Luminal A samples, (2) 37% of total 122 PAM50 Luminal B samples, (3) 47% of total 51 PAM50 Her2-enriched samples and (4) 33% of total 107 PAM50 Basal-like samples. In invasive lobular cancers, gain and amplification of *RAC1* were 24% of total 127 samples. Involvement of WP in different TNBC cells was tested following stimulation by LWnt3ACM and Wnt3A recombinant protein and different inhibitors of WP by both qRT-PCR and WB for beta-catenin, active beta-catenin, cMYC, cyclin D1 and WP specific several stem cell markers. The WP attenuation, which (a) decreased cellular levels of beta-catenin, as well as its nuclear active-form, (b) decreased fibronectin-induced migration & invasion, (c) altered actin dynamics and (d) decreased podia-parameters was successful in blocking fibronectin-mediated RAC1/Cdc42 activity. Both Wnt-antagonists and RAC1 inhibitors blocked fibronectin-induced RAC1 activation and inhibited fibronectin-induced ID-MA phenotypes following WP stimulation by LWnt3ACM and Wnt3A recombinant protein. High expression of RAC1 was associated with poor outcome for RFS with HR=1.48 [CI: 1.15-1.9] p=0.0019 in the Hungarian ER-ve BC cohort. **CONCLUSION:** In TNBC model, the activation of RAC1 signals downstream of WP mediated ID-MA phenotypes. The identification of the functional relationship between RAC1 signaling and the WP activation in the control of ID-MA mechanistically explains how the activation of WP in TNBC is associated with the high metastatic incidences and a dismal outcome.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-08-08

Title: GATA3 inhibits breast basal-like tumorigenesis

Pei X-H, Chan HL, Liu S, Scott A, Pimentel E, Slingerland J, Robbins D, Capobianco A and Bai F. University of Miami, Miami, FL.

Body: BACKGROUND: Breast cancer can be broadly categorized into two groups depending on the cell type affected. Luminal-type tumors are typically estrogen receptor (ER) positive that are associated with better survival and respond to hormone therapies whereas basal-like tumors are ER negative, more aggressive, and associated with a poor prognosis. GATA3 is a transcription factor well studied for its role as a master regulator of cellular differentiation and stem cell self renewal. Loss of Gata3 in mouse mammary glands blocks luminal cell differentiation and induces growth defects, and low levels of GATA3 are associated with basal-like and metastatic human breast cancers with epithelial-to-mesenchymal transition (EMT). Importantly, luminal cells have been shown to be the origin of some basal-like breast cancers. Due to the proliferation defects caused by GATA3 deficiency, it remains elusive how loss of function of GATA3 contributes to breast cancers development and progression.

METHODS: We previously demonstrated that p18^{Ink4c} (p18), a cell cycle inhibitor, is a downstream target of GATA3 in regulating mammary luminal cell proliferation and loss of p18 leads to luminal type tumorigenesis. To test the role of Gata3 deficiency in tumorigenesis, we generated *p18^{-/-};Gata3^{+/-}* mice. Mammary gland development and tumorigenesis were characterized *in vivo* using a panel of cellular and molecular assays. Results were further confirmed *in vitro* with well established cell lines.

RESULTS: Loss of p18 rescued mammary growth defects caused by Gata3 heterozygosity. Gata3 heterozygosity impaired luminal, but promoted basal gene expression in mammary epithelial cells. Gata3 heterozygosity in p18 null mice accelerated spontaneous mammary tumorigenesis, reducing the average latency of tumor onset. More importantly, Gata3 heterozygosity transformed the luminal type tumors of p18 null mice into heterogeneous basal-like breast cancers with activated EMT. Conversely, reintroduction of GATA3 inhibited tumor growth and reduced expression of EMT markers in basal-like tumor xenografts. We discovered that expression of GATA3 and Vimentin, an EMT marker, is inversely related in human breast cancers.

CONCLUSION: Our data indicates that GATA3 promotes luminal but suppresses basal cell differentiation in the mammary gland and in tumor development.

Mechanisms underlying the role of GATA3 in suppressing basal-like tumor development are under investigation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-08-09

Title: Cancer stem cells define 3D clonogenic growth response to rational combinations of PI3K-isoform specific inhibitors in TNBC

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Body: Introduction: 3D cell cultures have been recognized as the method of choice for physiologically relevant modeling of malignant behavior of tumor cells *ex vivo*. Morphologies of BC cell lines in 3D assays correlated with their gene/protein expression profiles reflecting underlying distinct morphologies associated with tumor cell invasiveness and metastases-associated phenotypes. Studies correlating patient outcome to CD44/CD24/CD44v6 expression highlight a need to understand the molecular characteristics of these cell populations to develop relevant therapies. Recently we have established a method to study cancer stem cell (CSC) populations following 3D clonogenic growth and demonstrated a characteristic pattern of CD44/CD24 expression based on mutational context (PTEN-null MDA-MB468, HCC70; RAS/RAF mutated MDA-MB231; BRCA mutated/PTEN-null SUM149 & HCC1937; PIK3CA mutated BT20) of TNBC cells (*AACR, 2016*) **Hypothesis:** Using 3D clonogenic growth of epithelial tumor cells to model CSC behavior *in vitro*, we hypothesized that there is a functional association between 3D clonogenic growth and CSC proportion following drug treatment. **Methods:** To study the role of CSCs in 3D clonogenic growth, tumor cells were treated with a PARP inhibitor and iso-form specific PI3K inhibitors for 7 days in 3D ON-TOP matrigel assay. Live cells were recovered following de-gelling using PBS-EDTA based buffer. Relative distribution of CD24/CD44/ CD44v6 expression was determined by flow cytometry and compared to cells growing in 2D format. **Results:** CD44/CD24/CD44v6 expression levels characteristically changed between 2D and 3D growth in individual cell lines. Combinations of AZD6482 and BMN673 was most effective in MDA-MB468 cells as it blocked proliferative signals and enhanced apoptosis as demonstrated by WB, flow cytometry, proliferation assay, cell viability, and live/dead cell assays. Combining GDC-0941 with BMN673 blocked proliferative signals and enhanced apoptosis in *PTEN*-null as well as *PTEN*-positive and RAS/RAF mutated TNBC cells. GDC0032 had limited effects in *PTEN*-null cells. In MDA-MB468 cells, control 3D-growth cells expressed a distinct CSC population as compared to their 2D counterpart. While BMN673 treatment increased CD44L/CD24H population cells as did GDC-0032 treatment, there were no alterations in this population following GDC-0941 or AZD6482. Combining AZD6482 plus BMN673 increased CD44L/CD24H population (67% from 22%) as compared to 3D-control and well as BMN673 single agent treatment (67% from 45%). Similar results were obtained with GDC0032 and BMN673 combination. In SUM149 cells 3D growth increased CD44L/CD24H population (from 23% to 83%) as compared to 2D growth, but drug treatment did not alter CSC distribution. No alternations were observed in MDA-MB231 cells. The mechanistic details of the effect of this combination on the CD44^H/CD44v6 stem cell populations are being worked out and will be presented at the meeting. **Significance:** Standardizing a method to determine CD24^L/CD44^H/CD44v6 CSC fraction from live cells following 3D clonogenic transformation, we determined functional correlation between CSCs and 3D clonogenic growth following a combination of PI3K isoform-specific inhibitor(s) and PARP inhibition.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-08-10

Title: Osteoprotegerin mediates tumor-promoting effects of Interleukin-1beta in breast cancer cells

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Body: It is widely recognized that inflammation promotes breast cancer invasion and metastasis. Given the complex nature of the breast tumor inflammatory microenvironment, much remains to be understood of the molecular mechanisms that govern these effects. We have previously shown that osteoprotegerin (OPG) knockdown in breast cancer cells resulted in reduced invasion and metastasis. Here we present novel insight into the role of OPG in inflammation-driven tumor progression in breast cancer cells by investigating the link between OPG and the potent pro-inflammatory cytokine IL1B (IL1B).

We used human breast cancer cell lines to investigate the effects of IL1B treatment on OPG expression. We analyzed public datasets containing human breast cancer genome-wide mRNA expression data to reveal a significant and positive correlation between OPG mRNA expression and the mRNA expression of IL1B as well as CCL2 (a monocyte chemoattractant protein). We determined the effect of macrophages (which produce IL1B) on OPG expression by co-culturing breast cancer cells and differentiated THP-1 macrophages. In order to demonstrate that OPG mediated functional effects of IL1B we performed cell invasion studies with control and OPG siRNA knockdown breast cancer cells treated with IL1B.

We report that OPG expression is induced by IL1B, independent of breast cancer subtype and basal OPG levels. Co-culture of breast cancer cells with IL1B-secreting macrophages resulted in a similar increase in OPG expression in breast cancer cells as IL1B treatment. We show that OPG expression is regulated by IL1B in a p38-dependent manner. We also demonstrate that OPG knockdown represses IL1B expression, IL1B-mediated breast cancer cell invasion and MMP3 expression.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-08-11

Title: Histone acetyltransferase 1 interacts with estrogen receptor alpha (ER α) and affects the transcriptional activity of ER α in breast cancer cells

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Body: Transcriptional regulation of estrogen receptor alpha (ER α) is a complex and multistep process. In order to identify novel proteins that are involved in ER α -mediated transcription, we used a quantitative proteomic method to identify cellular proteins that interact with ER α . Histone acetyltransferase 1 (HAT1) is one of the identified proteins. We have verified ER α -HAT1 interaction by performing coimmunoprecipitation and *in-vitro* binding assay. In addition, we found that the interaction occurred mainly in the nucleus. Domain mapping assay shows that ER α binds HAT1 primarily through the ligand binding E domain. In a luciferase assay, we found that knockdown of HAT1 by shRNA resulted in a ~5-fold increase in ER α -mediated transcription in breast cancer MCF7 cells, suggesting that HAT1 is functionally linked to ER α . An enzyme-dead mutant HAT1 showed similar effect on ER α transcriptional activity as the wild-type HAT1, suggesting that the enzyme activity of HAT1 is not involved in its effect on ER α transcriptional activity. Interestingly, Knockdown of HAT1 resulted in increased acetylation of histone H4 at lysine 8. Lastly, we demonstrate that the effect of HAT1 on ER α transcriptional activity is gene specific. Our data suggest that HAT1 regulates ER α -mediated transcription through affecting the interactions of ER α with histone proteins around the promoter region of ER α target genes in breast cancer cells.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-08-12

Title: DSCAM-AS1, a breast cancer specific and Estrogen receptor α -dependent long noncoding RNA, is a key component of the pathway controlling cell growth and migration

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Body: We previously reported that the long noncoding RNA (lncRNA) DSCAM-AS1 is one of the most interesting functional molecules in the Estrogen Receptor alpha (ER α) pathway in breast cancer cells, among a set of lncRNAs showing dependency on ER α in MCF7 cells (Miano et al., *Oncotarget* Jan 19, 2016. DOI: 10.18632/oncotarget.6420). Importantly, these lncRNAs were identified as dependent on ER α expression in absence of hormones, and DSCAM-AS1 was the most representative of them, presenting a clear ER α ChIP-Seq signal on the DSCAM-AS1 promoter and responding sharply to ER α silencing, but not to estradiol treatment. This behavior was shared with some other, but not all, ER α -dependent lncRNAs. We showed also that DSCAM-AS1 expression was strongly related to the luminal B > A tumor subtype and strongly related to ER+, in all datasets examined. Together with other lncRNAs we identified, they constituted a sharp luminal-specific gene signature. All this was confirmed more recently by another group (Niknafs et al., *Nat. Comm.* Sept 26, 2016. DOI: 10.1038/ncomms12791), also showing that DSCAM-AS1 may be related to endocrine resistance in breast cancer.

We report here that DSCAM-AS1 silencing in MCF7 cells evokes a response very similar to what we observed by knocking down ER α (Caizzi et al., *PNAS* 2009. DOI: 10.1073/pnas.1315445111), i.e. growth arrest, morphological changes and cell death. Noteworthy, ER α expression was not altered by DSCAM-AS1 silencing, thus indicating that DSCAM-AS1 is downstream ER α . Thus, we were interested in evaluating the overall transcriptional response to DSCAM-AS1 silencing. LNA-mediated DSCAM-AS1 down-regulation led to changes in the expression level of 436 protein-coding genes, as determined by RNA-seq and the following sample validation by qRT-PCR. Data analysis by means of IPA and EnrichR indicated that DSCAM-AS1 silencing regulated genes of cell growth and proliferation, cell signaling, cell death and survival and cellular movement. On the other side, there was also a clear stress response with involvement of the interferon signaling pathway. As in the case of ER α silencing, the overall picture is that DSCAM-AS1 may have a function in the maintenance of the luminal epithelial phenotype in breast cancer cells. Interestingly, genes related to cell movement were actually activated by DSCAM-AS1 knock-down and, in this respect, our result may be somehow contrasting with those shown by Niknafs et al. (above) who reported that stable DCAM-AS1 silencing by shRNA led to decreased migration and invasiveness. Differences in RNA-mediated long-term downregulation versus shorter term, LNA-mediated downregulation may account for discrepancies, but the matter clearly deserves more investigation. Finally, we present further data on the association of DSCAM-AS1 with ER α in breast tumors and clinical data. We suggest that its high level of expression, tissue-of-origin specificity and breast tumor phenotype specificity make DSCAM-AS1 an extremely interesting novel biomarker of luminal breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-01

Title: Predictors of locoregional recurrence after neoadjuvant chemotherapy and surgery for node positive breast cancer: Results from ACOSOG Z1071 (Alliance)

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Body: Background:

Locoregional recurrence (LRR) is a concern after neoadjuvant chemotherapy. Absolute risk of LRR and risk factors associated with LRR among women with node positive breast cancer treated with neoadjuvant chemotherapy on ACOSOG Z1071 were examined.

Methods:

ACOSOG Z1071 (Alliance for Clinical Trials in Oncology) enrolled cT1-3, N1-2 breast cancer patients treated with neoadjuvant chemotherapy from 2009-2011. All patients underwent axillary dissection. Data was analyzed for locoregional recurrence-free survival (LR-RFS) and multivariable analysis performed to identify factors impacting locoregional recurrence.

Results:

Of 756 women enrolled, 701 patients were eligible. Median follow-up was 4.0 years (range 0.03–6.2). 39 pts (5.6%) experienced LRR (32 LRR alone and 7 LRR concurrent with metastatic disease) and 96 patients (13.7%) died. LR-RFS was lowest in patients with triple negative tumors (TNBC) (87.6% at 3 years), followed by HER2-positive tumors (94.1%) compared with Hormone Receptor (HR) positive/HER2-negative tumors (96.9%).

Residual in-breast disease was present in 57% of patients undergoing BCT and 73% of patients undergoing mastectomy (p<0.0001). LRR was higher in BCT compared to mastectomy (p=0.018), however when evaluated by subtype there was no significant difference in LRR by BCT vs mastectomy. There was no difference in LRR by clinical T stage at presentation or pathologic N stage, however LRR increased with higher pathologic T stage (p=0.006).

Overall LRR was lower in patients who achieved a pathologic complete response (pCR) across all tumor subtypes (0% in HR positive/HER2-negative, 2.1% in TNBC, 4% in HER2-positive at 3 years) than in non-pCR patients (HR=0.37, p=0.04). By tumor subtype, pCR was associated with a lower LRR in patients with TNBC (HR=0.17, p=0.019), but was not significantly different by pCR in HER2-positive (HR=0.50, p=0.31) or HR-positive/HER2-negative tumors (HR=ineestimable).

In the multivariable model, factors associated with increased LRR risk were tumor subtype (TNBC and HER2-positive tumors, p<0.0001), lack of pCR in the breast (p=0.013), breast conservation surgery (p=0.018) and omission of adjuvant radiation therapy (p=0.010)

Predictors of locoregional recurrence

Variable	Univariable		Multivariable	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Clinical T stage				
T0-T2	REF	0.34	REF	0.087
T3-T4	1.38 (0.72-2.65)		1.87 (0.91-3.84)	
Tumor Biology				
HR+/Her2-	REF	0.002	REF	<0.0001
Her2+	1.49 (0.65-3.45)		2.11 (0.89-5.00)	
Triple negative	3.59 (1.68-7.68)		5.53 (2.53-12.13)	
Path CR breast				
No	REF	0.019	REF	0.013
Yes	0.35 (0.15-0.84)		0.27 (0.09-0.76)	

Path CR breast					
No	REF	0.064	REF	0.81	
Yes	0.51 (0.25-1.04)		0.67 (0.29-1.59)		
Surgery					
BCT	REF	0.067	REF	0.018	
Mastectomy	0.55 (0.29-1.04)		0.43 (0.22-0.87)		
Radiation					
Yes	REF	0.033	REF	0.010	
No	2.18 (1.06-4.48)		2.60 (1.26-5.39)		

Conclusion:

In patients with node-positive breast cancer treated with neoadjuvant chemotherapy, early LRR is higher in TNBC and HER2-positive tumors. In TNBC patients pCR is associated with lower LRR than residual disease. In this contemporary cohort of node-positive, HER2-positive tumors treated with anti-HER2 therapy, patients achieving a pCR had low LRR rate. Adjuvant radiation appears to be important for locoregional control regardless of pCR.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-03

Title: Impact of 70-gene signature use on adjuvant chemotherapy decisions in early breast cancer patients: Results of the prospective symphony triple A study

Kuijjer A, Straver M, Elias S, Smorenburg C, Wesseling J, Linn S, Rutgers E, Siesling S and van Dalen T. Diaconessenhuis, Utrecht, Netherlands; University Medical Center Utrecht, Utrecht, Netherlands; Netherlands Cancer Institute, Amsterdam, Netherlands and Netherlands Comprehensive Cancer Organisation, Utrecht, Netherlands.

Body: PURPOSE: gene-expression profiles, such as the 70-gene signature (70-GS), are increasingly used as adjunct to conventional clinicopathological prognostic factors to guide adjuvant chemotherapy (CT) decisions. The Dutch guideline suggests use of validated gene-expression profiles in estrogen-receptor (ER) positive (+) early stage breast cancer patients without overt lymph node metastases. We aimed to assess the impact of the 70-GS on CT decisions in ER+ early stage breast cancer patients. **PATIENTS AND METHODS:** In this prospective observational multicenter study physicians were asked for their opinion whether to administer or omit adjuvant CT before deployment and after obtaining the test result of the 70-GS in this guideline delineated group of patients.

RESULTS: Between January 1 2013 And December 31 2015 660 patients, treated in 31 hospitals, were enrolled. Based on the clinicopathological postoperative findings physicians would administer CT in 41%, withhold CT in 16% of patients and refrained from formulating an advice in the remaining 43% of patients letting their recommendation depend on the result of the 70-GS

Table 1. Concordance between pre-test CT recommendation of the oncologist and the 70-gene signature (GS) test result.

Pre-test CT recommendation	no. patients	70-GS test result	
		Low Risk	High Risk
No CT	107	63 (59%)	44 (41%)
CT	270	152 (56%)	118 (44%)
Depends on 70-GS result	283	174 (61%)	109 (39%)

Note. Agreement between pre-test oncologist CT recommendation and the 70-GS test result: Pearsons $r = -0,031$ 95%CI (-0,11 – 0,045).

. Estimated 5-year survival benefit of CT administration was 0.8%, 0.6% and 0.7% respectively ($p 0,585$). The 70-GS result hardly varied in relation to the initial advice of the physician: 56% and 59% had a low-risk profile in patients in whom CT was recommended or discommended respectively ($r=0.021$, Table 1). In 51% of patients in whom a pre-test recommendation was formulated incorporation of the 70-GS test result changed the initial advice. Adherence to the test result was high for the three groups (range 94-97%)

Table 2. CT recommendation before vs. after obtaining the 70-GS test result and the actual administration of CT.

Pre-test CT recommendation	no. patients	Post-test CT recommendation		Adherence to test result*	Actual administered CT		Adherence to test result*
		No CT	CT		No CT	CT	
No CT	107	69 (65%)	38 (35%)	94%	73 (68%)	34 (32%)	91%
CT	207	156 (58%)	114 (42%)	97%	162 (60%)	108 (40%)	90%
Depends on 70-GS result	283	173 (61%)	110 (39%)	95%	185 (65%)	98 (35%)	91%

*Percentage of patients in whom the post-test recommendation/actual administered CT was in line with the 70-GS test result (i.e. no CT in case of a low-risk profile and CT in case of a high-risk profile. Note. Change in CT recommendation in patients with a CT or no CT pre-test recommendation McNemar's chi-square test $p < 0.001$ and $p < 0.001$ for actual administered CT

CONCLUSION: guideline-directed use of the 70-GS in Dutch ER+ early breast cancer patients influenced CT treatment decision in the majority of patients. The physician's tentative CT advice was not associated with the 70-GS test-result.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-04

Title: Nuclear grade has a limited role in predicting recurrence in DCIS following breast conserving surgery: A population-based study

Nofech-Mozes S, Hanna W, Baehner FL L, Saskin R, Tuck A, Sengupta S, Elavathil L, Jani PA A, Bonin M, Chang MC C, Slodkowska E, Paszat L and Rakovitch E. Sunnybrook Health Sciences Centre, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; University of California, San Francisco, San Francisco, CA; Genomic Health, Inc., Redwood City, CA; Institute for Clinical Evaluative Sciences, Toronto, CA; London Health Sciences Centre, London, ON, Canada; Kingston General Hospital, Kingston, ON, Canada; Henderson General Hospital, Hamilton, ON, Canada; Thunder Bay Regional Health Sciences Centre, Thunder Bay, ON, Canada; Northern Ontario School of Medicine, Thunder Bay, ON, Canada; Health Sciences North Sudbury, Sudbury, ON, Canada and Mount Sinai Hospital, Toronto, ON, Canada.

Body: Aims: Clinical and pathologic characteristics including histologic grade are used in DCIS patients to assess biologic aggressiveness and the risk of ipsilateral breast recurrence (IBR) in order to align appropriate treatment. The landmark randomized DCIS trials that used an integrated assessment of grade that included nuclear grade, comedo necrosis and histologic subtype (Page 1987). Based on recent recommendations from the College of American Pathologists (CAP), histologic grade for DCIS is assessed by nuclear grade, separate from comedo necrosis and histologic subtype, (Lester, Arch Pathol Lab Med. 2009). ECOG E5194, a prospective cohort study of selected patients with low risk DCIS treated by wide excision and no radiotherapy, stratified patients using the former integrated DCIS grading system and found statistically significant differences in 7 year IBR rates between those with low/intermediate grade and high grade (Hughes, JCO 2009; Page 1987). When CAP nuclear grading was applied to 665 DCIS patients from the E5194 study, it was not associated with IBR (Solin, JCO, 2013 & 2015). The objective of this analysis is to evaluate whether CAP nuclear grade is an independent predictor of IBR in a population of patients with pure DCIS treated w/ breast conservative surgery (BCS).

Methods: Study cohort includes the Ontario population-based cohort of pure DCIS study of 3320 women with DCIS from 1994 to 2003 (Rakovitch, BCRT, 2015) REMARK guidelines were followed. Breast pathologists centrally reviewed all H&E slides for: focality (multifocality=at least 2 foci of DCIS 5mm apart), size, CAP nuclear grade (Lester, Arch Pathol Lab Med. 2009), histologic subtype, comedo necrosis & clear margins, (CM=no ink on tumor). Cox modeling was used to determine the relationship between independent covariates & IBR.

Results: Tumor blocks were collected for 1751 patients (53% of parent cohort); 718 treated with BCS alone (N=571 with CM). Median follow-up was 9.6 years. Among the 571 pts with CM, 541 were ER+ and 100 patients had an IBR (DCIS, N=44; invasive, N=57). Distribution of nuclear grade was: 55 (9.6%) low nuclear grade, 332 (58.1%) intermediate nuclear grade, and 184 (32.2%) high nuclear grade. The 10-year rates of developing an IBR were: low grade 15.2% (7.9-28.1); intermediate 16.5% (12.7-21.3%), high 25.6% (19.3-33.6), respectively (log rank P=0.1006). In multivariable analyses for IBR in CM cases including nuclear grade (H vs. L/I), comedo necrosis, histologic subtype, tumor size, age and focality only multifocality (P=0.0002), histologic subtype P=0.016), size (P=0.028) and age ≥ 50 (P=0.018) were significant.

Conclusions: CAP nuclear grade as assessed by central pathology review was not significantly associated with the risk of IBR in DCIS patients treated with BCS alone from the Ontario population based DCIS study. These findings confirm those observed in E5194. CAP nuclear grade results in grade migration and is not informative in prediction of IBR. Further studies to determine whether appropriate assessment of grade should return to the integrated grading that was used and was informative in the landmark randomized radiotherapy trials are required.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-05

Title: Prognostic and predictive values of high endothelial venules (HEV) and tumor infiltrating CD8+ lymphocytes (CD8) in tumors of patients included in the adjuvant PACS04 trial: HEV is predictive of outcome for HER2+ tumors exposed to trastuzumab

Roché HH H, Lafouresse FF F, Filleron T, Laffont R, Maisongrosse V, Pichery M, Le Guellec S, Penault-Llorca F, Lemonnier J, Lacroix-Triki M and Girard J-P. Institut Claudius Regaud - IUCT-O, Toulouse, France; IPBS-CNRS Université, Toulouse, France; Centre Jean Perrin, Clermont-Ferrand, France and R&D Unicancer, Paris, France.

Body: Background: HEV are specialized blood vessels that function as portals of entry for lymphocytes into lymphoid organs and tumor tissues (Moussion and Girard, Nature 2011, 479:542-546; Girard et al, Nature Rev Immunol 2012, 12:762-773). We retrospectively considered HEV and CD8 as potential prognostic and/or predictive factors in a large randomized adjuvant trial of node positive breast cancer patients (PACS04). This trial included 3010 node positive patients randomized between anthracyclins alone or anthracyclins and docetaxel chemotherapy. Patients with HER2+ expressing tumors had a second randomization with or without trastuzumab given sequentially for one year. With 59.5 median follow-up, metastatic free interval (MFI), the first end-point, was 84.5% at 5 years for the whole population.

Methods: 1660 tumor samples (9.7% triple negative, 67.5% HR+/HER2- and 22.8% HER2+) were collected and analyzed by immunostaining on full sections for HEV (MECA-79 mAb, BD Biosciences) and CD8 (C8/144B mAb, Dako). HEV densities were determined as previously described (Martinet et al., Cancer Res 2011, 71:5678-5687). CD8+ cells and tumor-infiltrating lymphocytes (TIL) were scored according to recently published guidelines. Univariate analyses were performed using cox proportional hazard model for continuous variable. Independent analyses for the predictive evaluation of trastuzumab outcome were performed in the HER2+ subgroup.

Results: MFI and overall survival at 5 years for this series are respectively of 84.9% (TN: 77.4%, HR+/HER2-: 89%, HER2+:75.8%) and 91% not different with the total group. The table shows expression of the different markers according to the subgroup of tumors.

Marker values according to sub molecular classification

	TN	RH+/HER2-	HER2+/RH-
Number	160	1119	378
Metastatic events	34	119	90
HEV/mm ² (median,range)	0.51 (0, 7.73)	0.13 (0, 10.23)	0.38 (0, 13.63)
CD8score (median, range)	2 (0, 3)	1 (0, 3)	2 (0, 3)

Table 1

No difference in univariate analysis was observed in TN and HR+/HER2- subgroups in terms of relationship between marker expression and outcomes. For the HER2+ group, HEV and CD8 were correlated to better outcome (HEV: HR=0.73, p =0.011; CD8: HR=0.64; p=0.006). For HER2+ patients not receiving trastuzumab (222 pts, 55 events), CD8 was predictive of metastasis risk (HR: 0.65, p=0.032), but not HEV (HR:0.82, p=0.09). Conversely, in the trastuzumab treated group (156 pts, 35 events), HEV was significantly correlated with a lower risk of relapse (HR: 0.45, p=0.02), but CD8 was not (HR:0.63, p=0.07). TIL counts are still ongoing and will be reported at time of presentation.

Conclusions: HEV and CD8 are associated with better prognosis in the HER2+ tumor group. Interestingly, HEV presence in the tumor seems to be a significant predictive factor of trastuzumab efficacy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-06

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-07

Title: Mutation characteristics and tumor infiltrating lymphocytes in early and metastatic HER2-positive breast cancer

Kotoula V, Giannoulatou E, Kouvatseas G, Tikas I, Lazaridis G, Charalambous E, Efstratiou I, Bobos M, Tsolaki E, Zagouri F, Christodoulou C, Pentheroudakis G, Koutras A, Papakostas P, Kosmidis PA A, Pectasides D and Fountzilas G. Hellenic Cooperative Oncology Group (HeCOG), Athens, Greece; Victor Chang Cardiac Research Institute, Darlinghurst, NSW, Australia and Health Data Specialists Ltd, Athens, Greece.

Body: Background-aim: HER2-positive breast cancer (BC) features high rates of tumor infiltrating lymphocytes (TILs) and mutations (mut) in various genes, more frequently in TP53. We investigated associations between TILs and mutations in HER2-positive BC and their impact on patient outcome in early and metastatic BC (EBC and MBC, respectively), which remain largely unexplored.

Methods: In 352 primary paraffin tumors from patients with HER2-positive disease, we examined amino acid changing mutations (<0.1% minor allele frequency) in 58 genes for type and possible clonality (>20% variant frequency). Study groups were: (A) 218 EBC, including 117 patients treated with adjuvant chemotherapy only (CT) and 101 patients treated with CT and trastuzumab (CTT); (B) 134 MBC, including 95 patients who relapsed upon adjuvant CT without trastuzumab (R-MBC) and 39 patients who were first diagnosed with metastatic disease (de novo MBC). TILs were assessed as percentage of stromal tumor area. Clinical endpoints were disease-free survival in 5 years (5yr DFS) for EBC, and time-to-progression (TTP) from 1st line CTT treatment start for MBC.

Results: 243/352 tumors (69%) carried at least one mut; 27/352 (8%) of tumors >10 up to 150 mut (hypermut); 192/352 (54%) at least one possibly clonal mut. Mean mut number and TP53 mut in particular were highest in R-MBC and lowest in EBC; mean TILs density followed the opposite pattern (all $p < 0.001$). TILs density was lower in all settings in hypermut tumors and in tumors with multiple clonal mut (p values 0.043 – 0.050). Upon multivariate analysis in EBC, higher risk for relapse in 5yrs was noticed for CT patients compared to CTT (odds ratio [OR] 2.39, 95%CI [CI] 1.13-5.04, $p=0.023$) and for >3 compared to 0-3 positive nodes (OR 3.83, CI 1.76-8.34, $p=0.001$); lower risk for relapse was observed for higher TILs irrespectively of treatment (OR 0.93, CI 0.90-0.97, $p=0.001$), for TP53 mut (OR 0.39, CI 0.18-0.87, $p=0.022$) and for clonal TP53 mut in CTT-treated patients (OR 0.10, CI 0.02-0.58) but not in CT-treated patients (interaction $p=0.084$). The presence of any clonal mut (hazard ratio [HR] 2.77, CI 1.42-5.38) and of clonal TP53 mut (HR 2.24, CI 1.20-4.17) conferred worse TTP in de novo but not in R-MBC; these interactions remained significant upon multivariate analysis (interaction $p=0.007$ and $p=0.061$, respectively). Higher TILs in the absence of clonal mut conferred longer TTP (HR 0.75, CI 0.56-0.99) but no such effect was observed for tumors with clonal mut (multivariate interaction $p=0.052$). Classic independent predictors of unfavorable TTP in MBC were younger age ($p=0.002$), absence of hormone receptors ($p=0.001$) and poor performance status ($p=0.044$). PIK3CA mut did not remain significant in any of the examined settings.

Conclusions: The expected pattern of higher TILs associated with mutation number and clonality was not observed in HER2-positive BC; the favorable effect of TILs only in the absence of clonal mut in MBC may imply exhausted immune response. Clonal TP53 mut may serve as a predictor for trastuzumab benefit in EBC but as an adverse prognosticator in trastuzumab-treated de novo MBC, which, if further validated, is of potential clinical relevance.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-08

Title: Real-world clinical experience and outcomes in patients with early-stage breast cancer (EBC) treated according to the 21-gene recurrence score® (RS) result

Sing AP P, Rothney M, Svedman C, Shak S and Baehner FL L. Genomic Health, Inc., Redwood City, CA.

Body: Evaluating the merits of a genomic assay includes measuring analytic and clinical validity, and establishing clinical utility—a property that is not consistently defined. One definition of clinical utility that has gained traction is “the balance of benefits and harms associated with the use of the test in practice” [*Genet Med.* 2015; doi:10.1038/gim.2015.173].

The 21-gene RS assay is the only assay clinically validated for both prognosis and prediction of chemotherapy (CT) benefit in patients with node-negative (N0) or node-positive (N+), ER+, HER2– EBC. The original clinical validation studies were prospectively designed using archived tissue from legacy trials that had long-term outcomes (NSABP B-14 and B-20, TransATAC, and SWOG 8814). With 10+ years of the RS assay in clinical use, we now have real-world, prospective outcomes for patients with N0 or N+ disease that meet the aforementioned definition of clinical utility.

Here we summarize the growing body of clinical evidence including the original validation studies, prospective outcomes-based trials, and analyses from two large, real-world registries in which patients were treated based on RS results: the US SEER and Clalit Health Services registries.

The US SEER registry is a population-based cancer surveillance program that covers 30% of the US population and includes 40,134 N0 and 4,691 N+ patients with RS results. The Clalit Health Services registry, from the largest HMO in Israel, has 2,028 N0 and micrometastatic (Nmi) patients who were uniformly tested and had complete treatment information. In the SEER and Clalit cohorts, the distribution of RS results were similar for N0/Nmi and N+ patients: 54% and 57% low (<18), 38% and 36% intermediate (18-30), and 8% and 7% high (≥31), respectively. 5-year outcomes by RS group show that patients with low RS results of any nodal status (N0/Nmi/N1-3) had similar outcomes (Table). Outcomes by RS group and age or grade were also similar for patients with low RS results of any nodal status.

5-y BC-specific Mortality (95% CI)

	SEER Registries				Clalit Registry	
	N0 ^a (N=38,568)	Known CT use (% of N)	N+ ^b (N=4,691)	Known CT use (% of N)	N0/Nmi ^c (N=2,028)	Known CT use (% of N)
RS <18	0.4% (0.3%-0.6%)	7%	1.0% (0.5%-2.0%)	23%	0.0% (0.0%-0.0%)	2%
RS 18-30	1.4% (1.1%-1.7%)	34%	2.3% (1.3%-4.1%)	47%	1.1% (0.5%-2.1%)	25%
RS ≥31	4.4% (3.4%-5.6%)	69%	14.3% (8.4%-23.8%)	75%	6.8% (4.1%-11.2%)	88%

a. Includes patients 40-84 years of age only (HR+, HER2–, nonmetastatic EBC). b. Includes Nmi and up to three positive nodes [N+(mi,1-3)]. c. Includes 1,815 (89%) N0 and 213 (11%) Nmi.

In summary, after 10+ years of clinical use, the 21-gene RS assay has now amassed a body of clinical evidence from >50,000 patients that confirms the original clinical validation results and supports its clinical utility. The assay identifies patients with low RS results who can be safely and effectively treated with hormonal therapy alone and spared the toxicity of CT exposure. In aggregate, these data support the clinical utility of the 21-gene RS assay and its value to physicians and patients by providing information based on individual tumor biology that they can use to tailor treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-09

Title: Evaluation of tumor infiltrating lymphocytes (TILs) and their association with homologous recombination deficiency and *BRCA1/2* mutation status in triple-negative breast cancer (TNBC): A pooled analysis

Telli ML L, Badve S, Vinayak S, Silver DP P, Isakoff SJ J, Kaklamani VG G, Gradishar WJ J, Stearns V, Connolly RM M, Ford JM M, Adams S, Garber JE E, Evans B, Timms K, Wenstrup R and Richardson AL L. Stanford University School of Medicine, Stanford, CA; Indiana University, Indianapolis, IN; Case Western Reserve University School of Medicine, Cleveland, OH; Dana Farber Cancer Institute, Boston, MA; Massachusetts General Hospital, Boston, MA; Harvard Medical School, Boston, MA; UT Medicine Cancer Therapy and Research Center, San Antonio, TX; Northwestern University, Chicago, IL; Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore, MD; New York University Cancer Institute, NYU School of Medicine, New York, NY and Myriad Genetics, Inc., Salt Lake City, UT.

Body: Background: TNBC patients with homologous recombination (HR) deficient tumors have significantly higher pathologic complete response (pCR, ypT0/is ypN0) rates when treated with platinum-based chemotherapy. TILs are prognostic and predictive of chemotherapy benefit in TNBC. Interestingly, recent data suggests that HR deficient TNBCs and *BRCA1/2* mutant ovarian cancers may be enriched for immune cell infiltration. Thus, we performed a pooled analysis of 5 phase II studies that included patients with TNBC treated with neoadjuvant platinum-based chemotherapy to evaluate the association of TILs with HR deficiency status and tumor *BRCA1/2* mutation status.

Methods: 166 patients with TNBC and known HR deficiency status from the following clinical trials were available for analysis: PrECOG 0105 (N=72), NCT00580333 (N=32), NCT01372579 (N=26), TBCRC 008 (N=18) and NCT00148694 (N=18). Neoadjuvant chemotherapy regimens included 1) carboplatin, gemcitabine, iniparib, 2) cisplatin with or without bevacizumab 3) carboplatin, eribulin, 4) carboplatin, nab-paclitaxel, with or without vorinostat. HR deficiency status was defined as a high HRD score (42 or higher) and/or presence of a *BRCA1/2* tumor mutation (tBRCA). Digitized pre-treatment core biopsy H&E sections were reviewed and scored by two blinded expert breast cancer pathologists using the international TIL working group guidelines. The density (%) of both intratumoral TILs (iTILs) and stromal TILs (sTILs) was recorded by deciles (n=122 thus far, additional cases to be included in final analysis).

Results: Among 122 patients, pCR was achieved in 36 patients (29.5%) and 71 tumors (58.2%) were HR deficient. In total, 24 patients (19.7%) patients had a deleterious *BRCA1/2* mutation. Among all tumors, iTIL and sTIL median densities were 0% (range 0-20) and 10% (range 0-80), respectively, and were not statistically different in HR deficient versus non-deficient tumors (iTIL p=0.746; sTIL p=0.159). The same absent association was observed for tBRCA mutation status (iTIL p=0.607; sTIL p=0.315) and binary HRD score (iTIL p=0.879; sTIL p=0.364). Updated results with additional cases scored for TILs will be reported at time of presentation. Additional analyses assessing the relationship between TILs and pCR/residual cancer burden adjusting for HRD status and other clinical variables will also be included at the time of presentation.

Conclusion: Several previous studies have reported that TILs are significantly associated with response to standard chemotherapy regimens when given in the neoadjuvant setting. Measures of genomic instability and DNA repair deficiency, including HRD, have been shown to be robust predictors of response to chemotherapy, including platinum containing regimens. It is unclear if TILs will be predictive in a similarly robust way for response to platinum-containing regimens. Results of this study suggest TIL density and HR deficiency status may be independent and non-overlapping. Final analysis including additional cases will be included in the final presentation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-10

Title: Results of multigene assay (MammaPrint®) and molecular subtyping (Blueprint®) substantially impact treatment decision making in early breast cancer: Final analysis of the WSG PRIME decision impact study

Wuerstlein R, Gluz O, Kates R, Persoon M, Wasmayr M, Knauer M, Koplmüller R, Grischke E-M, Schem C, Thill M, Hasmueller S, Griesinger F, Koehler A, Otremba B, Schindlbeck C, Reimer T, Krauter J, Tome O, Otto F, Friedrichs K, Albert U-S, Gebauer G, Nitz U and Harbeck N. University Hospital Munich, CCC Munich, Munich, Germany; WSG, Moenchengladbach, Germany; Breast Center Niederrhein, Moenchengladbach, Germany; Agendia, Amsterdam, Netherlands; Others, Germany; Others, Austria and Others, Switzerland.

Body: Background: In luminal early breast cancer (EBC) with limited nodal involvement, current guidelines recommend to use multigene assays in addition to conventional clinicopathological factors for decision making regarding adjuvant chemotherapy (CT). The WSG PRIME Study prospectively evaluated the impact of the 70-gene signature (MammaPrint®) and the corresponding molecular subtype (Blueprint®) on clinical therapy decisions in EBC.

Methods: WSG PRIME recruited 452 consecutive patients (pts) in 34 centers with ER+ and/or PR+ HER- pN0-1 EBC (04/15-03/16). Of the 430 evaluable pts, 309 had pN0 and 121 pN1 disease; median age was 58 years (68% post-menopausal). MammaPrint®, TargetPrint®, and Blueprint® results were provided prospectively, after therapy decisions based on clinicopathological factors and/or local IHC (ER/PR/Ki67) had been ascertained.

Results: The WSG PRIME study observed a switch in CT decisions based on the multigene test results of 28.4 % (95% CI 23.3-33.4%). In 57 pts (13.3%), the decision switched from CT to no-CT, in 65 (15.1%) from no-CT to CT; in 107 (24.9%) pts a CT decision and in 201 (46.7%) a no-CT decision was maintained. Physicians strongly adhered to test results, most notably when initial CT recommendation was discordant with the test: 62/72 (86.1%) switched from no-CT to CT following MammaPrint® high risk, with 84.7% in N0 and 92.3% in N1. For MammaPrint® low risk, 56/80 (70%) switched from CT to no-CT; this percentage was similar in pN0 and pN1. Overall adherence (all pts) was 92.9% (CT) for high risk and 90.1% (no-CT) for low risk. Regarding subtype, 1/430 tumors was classified as HER2-enriched by Blueprint®; of the 6 basal-like tumors by IHC, 2 were molecularly re-classified as luminal A and 4 as luminal B. Of the 424 luminal-A/-B-like tumors by IHC, only 283 (66.7%) were subtyped concordantly by Blueprint®; 40% (61/152) of the luminal-B-like tumors were re-classified as luminal A and 29% (79/272) of the luminal-A-like tumors were re-classified as luminal B. Switches in CT decisions were strongly impacted by molecular subtype, with 142/152 (93.4%) of molecular luminal B pts eventually scheduled for CT and 247/272 (90.8%) of molecular luminal A pts for no-CT. After the test results, physicians' confidence in their therapy decision increased in 141 (32.9%) of the cases.

Conclusions: In a decision impact study, the true percentage of CT saved by a prognostic test depends on cohort characteristics. Yet, the WSG PRIME study showed that the 70-gene signature (MammaPrint®) and the corresponding molecular subtype (Blueprint®) strongly impacted clinical therapy decisions in EBC with up to 3 involved lymph nodes. There was a substantial discordance between clinical and molecular luminal subtypes. After receiving the test results, physicians focused their indications for adjuvant CT on MammaPrint high-risk luminal-B-like tumors. Where test results were discordant with clinical assessment, physicians mostly omitted CT in MammaPrint low risk patients. Recently, the prospective MINDACT trial had shown that this did not compromise outcome.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-11

Title: Genetic variation in CYP3A affects steady-state exemestane concentrations but does not explain inter-race difference

Hertz DL L, Kidwell KM M, Gersch CL L, Desta Z, Storniolo AM, Stearns V, Skaar TC C, Hayes DF F, Henry NL and Rae JM M. University of Michigan, Ann Arbor, MI; Indiana University and Johns Hopkins University.

Body: Background: Exemestane is a third generation steroidal aromatase inhibitor (AI) used for the treatment of estrogen receptor (ER) positive breast cancer in postmenopausal women. Differences in AI treatment efficacy and side effects may be due, in part, to variability in drug exposure. We previously reported that patients who self-report as white and those who carry the low-activity CYP3A4*22 single nucleotide polymorphism (SNP) have increased exemestane steady-state concentrations. Additional SNPs in CYP3A may contribute to pharmacokinetic variability and explain this inter-race difference. CYP3A5*3 (rs776746) is a non-expressor genotype that is far more common in European (minor allele frequency (MAF)~0.94) than African (MAF~0.18) individuals. CYP3A7*1C (rs45446698) is believed to tag adult expression of the fetal CYP3A7 enzyme and is relatively uncommon in tested cohorts (European MAF=0.04, African MAF<0.01). The objective of this secondary analysis was to determine whether these additional CYP3A SNPs contribute to variability in steady state exemestane concentrations and explain the inter-race difference.

Methods: 500 patients were randomly assigned to either drug on the Exemestane and Letrozole Pharmacogenetics (ELPh) Study. Clinical data and DNA were collected at baseline and blood samples were collected after 1 or 3 months of treatment to measure steady-state exemestane concentration via HPLC/MS. Genotyping for CYP3A5*3 and CYP3A7*1C was performed via Taqman Allelic Discrimination. Pharmacogenetic association with log-transformed concentrations were tested for each variant by inclusion in a multivariable model with CYP3A4*22 and self-reported race, assuming additive genetic effect, using Tobit regression to censor concentrations below the lower limit of quantification. SNPs with suggestive p-values <0.10 were included in a multivariable model with relevant covariates (AST or ALT>40, body mass index (BMI), and prior chemotherapy) to assess their independent contribution.

Results: In 231 evaluable patients there was a suggestive trend toward lower steady-state exemestane concentrations for CYP3A7*1C carriers (6.3 vs. 8.0 ng/mL) in the model including CYP3A4*22 and race (p=0.083). In the final multivariable model each CYP3A7*1C allele decreased exemestane concentration 31.5% (p=0.035, Table 1). CYP3A5*3 was not associated with exemestane concentration (p>0.2).

Multivariable Model of Exemestane Concentration

	% change in concentration (95% CI)	p-value
CYP3A4*22 (rs35599367)	64.5% (23%, 120%)	0.0008
CYP3A7*1C (rs45446698)	-31.5% (-52%, -2.6%)	0.035
Self-Reported White	47.2% (9.0%, 99%)	0.012
AST or ALT>40	41.3% (1.0%, 98%)	0.044
BMI	-0.9% (-2.4%, 0.55%)	0.22
Prior Chemotherapy	-23.5% (-37%, -7.6%)	0.006

CI: Confidence Interval

Conclusions: Patients with breast cancer who carry CYP3A7*1C have lower steady-state exemestane concentrations but this association does not explain the greater concentrations in self-reported white patients. Ongoing analyses will determine whether exemestane concentration predicts treatment efficacy or toxicity, and if so, whether genetic and clinical factors can be useful for individualizing dosing to optimize outcomes. CYP3A7*1C should be prioritized for analyses of pharmacokinetic variability of other CYP3A substrates.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-12

Title: A consecutive series of early breast cancers with a low estrogen receptor expression

Paredis D, Wildiers H, Vergote I, Smeets A, Van Limbergen E, Remmerie C, Laenen A, Jongen L, Floris G and Neven P. KU Leuven - University of Leuven, University Hospitals Leuven, Leuven, Vlaams-Brabant, Belgium; KU Leuven - University of Leuven, University Hospitals Leuven, Leuven, Vlaams-Brabant, Belgium; KU Leuven - University of Leuven, University Hospitals Leuven, Leuven, Vlaams-Brabant, Belgium; KU Leuven - University of Leuven, University Hospitals Leuven, Leuven, Vlaams-Brabant, Belgium; KU Leuven- University of Leuven, University Hospitals Leuven, Leuven, Vlaams-Brabant, Belgium; Interuniversity Centre for Biostatistics and Statistical Bioinformatics, Leuven, Vlaams-Brabant, Belgium and KU Leuven - University of Leuven, University Hospitals Leuven, Laboratory of Translational Cell & Tissue Research and University Hospitals Leuven, Leuven, Vlaams-Brabant, Belgium.

Body: Background: Estrogen receptor (ER) positivity is not a dichotomous biological phenomenon and within the ER positive cohort, major differences in amount and percentage of ER expression are observed. Women with tumors expressing low ER levels have worse outcome as quantitative ER (qER) expression is predictive for benefit from hormone therapy. The aim of our study was to further characterize these patients, tumors and the distant relapse free survival (D-RFS) based upon qER expression.

Patients and Methods: In this retrospective study we included women with primary operable human epidermal growth factor receptor 2 (HER2) negative breast cancers, diagnosed between January 1st 2000 and September 30th 2015 in the Multidisciplinary Breast Center of University Hospital of Leuven. ER-low tumors ((1-33% immunohistochemical (IHC)staining, Allred proportion score (2-3/5), or H-score (<100/300 + <33% staining)) were compared with ER-high tumors(> 33% IHC staining, Allred proportion score (4-5/5), or H-score \geq 100/300)) and triple negative (TN) tumors (Allred proportion score (0-1/5) or H-score 0% staining). PR and HER2 were scored according to ASCO/CAP guidelines. Local and adjuvant therapies were given according to the in house guidelines. The Fisher exact test was used for comparing groups on categorical variables and the Kruskal Wallis test or Mann-Whitney test were used for continuous variables. The Kaplan-Meier method was used for estimating the interval to relapse. The log rank test was used for the comparison between groups (2-sided test with 5% significance, SAS software version 9.4).

Results: A total of 5390 women were included, 115 (2 %) were ER-low, 4658 (86.5%) were ER-high and 617 (11.5%) were TN. Average follow-up was 6 years. Patients with ER-low and TN breast tumors were younger at diagnosis compared to patients with ER-high tumors (respectively 55.5y and 56.7y vs 59.3 y; $p < 0.001$). Tumor size and nodal status did not differ between the three subgroups. ER low and TN were more often grade 3 (61% and 89% vs 24%, $p < 0.001$) which led to a higher mean NPI. Adjuvant chemotherapy was more likely to be given in ER-low and TN cases (57% and 75% vs 26%, $p < 0.001$). Women with an ER-low and TN breast cancer were more often referred for BRCA1/2/CHEK-2 testing as compared to those with an ER-high tumor (respectively 21%, 22% vs 10% $p < 0.001$); a mutation was found 5 to 7 times more in the ER low and TN group (5%-7% vs 1% $p < 0.001$). Metastatic relapse was 18% in ER low (21/114 patients), 15% in TN (90/617 patients) and 6% in ER high (283/4658 patients). Using a Kaplan Meier curve, the 5 year D-RFS was 84%, 85% and 96% in ER-low, TN and ER-high cases. The 10 year D-RFS was respectively 76%, 84% and 91% ($p < 0.001$).

Conclusion: ER-low breast cancers are rare and correlate better with TN than ER-high tumors regarding demographics, tumor grade, BRCA1/2/CHEK-2 mutation risk and breast cancer outcome.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-13

Title: The association between pCR status after neoadjuvant chemotherapy and sites of first distant relapse after surgery: A substudy of the EORTC 10094/BIG-1-00 trial

Touati N, Aalders K, Slaets L, Tryfonidis K, Cameron DA A and Bonnefoi H. European Organization for Research and Treatment of Cancer, Brussels, Belgium; European Organization for Research and Treatment of Cancer, Brussels, Belgium; Western General Hospital & Edinburgh University, Edinburgh, United Kingdom and Institut Bergonié, University de Bordeaux, Bordeaux, France.

Body: Background

Chemotherapy in eligible patients is increasingly applied in the neoadjuvant setting, as it offers the possibility for 'in vivo' monitoring of the activity to the administered treatment, prevention of early micrometastatic spread and often allows less invasive surgery in patients that would otherwise have needed a mastectomy. The achievement of a pathologic complete response (pCR) after neoadjuvant chemotherapy is a prognosticator for better outcome. However, less is known about the patterns of distant relapse between patients that did and did not achieve pCR. We assessed the differences in sites of first distant relapse after pCR versus non-pCR after neoadjuvant chemotherapy in patients enrolled in the EORTC 10094/BIG-1-00 "p53" trial.

Methods

The analyzed population consisted of patients enrolled in the "p53" trial that received ≥ 1 cycle of chemotherapy before surgery and who have been diagnosed with a distant relapse. pCR was defined as no evidence of residual invasive cancer (or very few scattered tumor cells) in the primary tumor and axillary lymph nodes with or without residual ductal carcinoma in situ (DCIS). Intrinsic subtype classification was performed using the 2011 St Gallen consensus. The first site of distant relapse was collected for all patients and was classified as soft tissue, visceral, skeletal or CNS. As primary analysis, the associations between achievement of pCR and sites of distant relapse were investigated in 4 multivariate logistic regression models, one for each site, adjusting for intrinsic subtype and preceding local recurrence (yes/no). Adjusted P-values are reported (Benjamini-Hochberg correction). Secondary analyses include: associations between site of first distant relapse and pCR by subtype, description of concomitant sites of relapse.

Results

The study included 383 (21%) eligible patients out of the 1856 randomized for the 'p53' trial, of whom 28 (7%) had achieved pCR and 355 (93%) did not. Median follow-up was 5.4 years. Achievement of pCR was associated with a trend towards a decreased presentation with skeletal metastases (21% (pCR) vs 50% (non-pCR), OR=0.32, adj-p=0.071, see Table) and we observed an increase in the proportion of patients with CNS tissue as first site of distant relapse (21% vs 9%, OR=2.39, adj-p=0.183). The trend for skeletal metastases was seen in all subtypes except for Luminal A. Patients with pCR were more likely to present with only one relapse location category when compared to non-pCR (86% vs 69%).

Association between site of first distant relapse and pCR

Site of first distant relapse	Non-pCR N=355	pCR N=28	Total N=383	Median of time from surgery till first distant relapse	pCR: Yes vs No	
Category	N(%)	N(%)	N(%)	Months	OR (95% CI)	Adj. p-value
Soft tissue	43 (12.1)	4 (14.3)	47 (12.3)	25	0.94 (0.30, 2.95)	0.909
Visceral	183 (51.5)	14 (50.0)	197 (51.4)	22	0.80 (0.36, 1.74)	0.756
Skeletal	179 (50.4)	6 (21.4)	185 (48.3)	28	0.32 (0.12, 0.82)	0.071
CNS	32 (9.0)	6 (21.4)	38 (9.9)	16		0.183

					2.39 (0.87, 6.58)	
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Conclusion

In this group of patients treated with neoadjuvant chemotherapy, patients that achieved a pCR were less likely to present with skeletal metastases as first site of distant relapse, even after adjustment for intrinsic subtype.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-14

Title: Prognostic significance of a modified residual disease in breast and nodes (mRDBN) algorithm after neoadjuvant therapy for breast cancer

Farrugia DJ J, Landmann A, McAuliffe PF F, Diego EJ J, Johnson R, Bonaventura M, Soran A, Dabbs DJ J, Clark B, Lembersky BC C, Puhalla SL L, Brufsky A, Jankowitz R, Davidson NE E, Ahrendt GM M and Bhargava R. University of Pittsburgh Medical Center, Pittsburgh, PA.

Body: Background: Patients achieving pathologic complete response to neoadjuvant chemotherapy have excellent disease free and overall survival. For patients with residual disease, the residual disease in breast and lymph node (RDBN) method provides useful prognostic information. RDBN is calculated as follows: $0.2 \times \text{tumor size (in cm)} + \text{lymph node status (0-3)} + \text{tumor grade (1-3)}$. pCR, low, intermediate and high risk of recurrence categories correspond to RDBN index of 0, 0.1 to 2.9, 3 to <4.4, and ≥ 4.4 , respectively. We hypothesized that the prognostic accuracy of RDBN may be improved by also taking into account the residual tumor cellularity.

Methods: Retrospective review of 614 consecutive patients who underwent neoadjuvant therapy for breast cancer was performed. At our institution, tumor size/volume reduction in the breast is determined using the equation:

Estimated % tumor size reduction = $[(\text{pre-therapy clinical size} - \text{“revised” pathology tumor size}) / \text{pre-therapy clinical size}] \times 100$.

“Revised” pathology tumor size is calculated by multiplying the largest dimension of the gross tumor bed by the invasive tumor cellularity of the tumor bed (in comparison to the pre-therapy core biopsy sample). For example, if a 3 cm tumor bed has only 50% cellularity for invasive cancer (in comparison to pre-therapy core biopsy), the revised tumor size is 1.5 cm. Hence, we were able to use the “revised tumor size” for calculating the modified RDBN index (mRDBN). We also used gross tumor bed size for gross RDBN (gRDBN) to compare with mRDBN. mRDBN and gRDBN could be calculated on 459 of the 514 cases. Chi-Square statistical analysis was performed.

Results: Mean follow up was 33.1 months (median 31, range 4-70).

The results are shown in Table 1 & 2.

Table 1. Overall Recurrence and Mortality

RDBN Score Category	Overall Recurrence				Mortality		
	n	n (%)	RR	95% CI; p	n (%)	RR	95% CI; p
mRDBN (n=459)							
High	58	29 (50.0)	19.63	[7.22, 53.40]; p<0.0001	18 (31.0)	16.24	[4.97, 53.10]; p<0.0001
Intermed	164	33 (20.1)	7.9	[2.86, 21.78]; p<0.0001	15 (9.1)	4.79	[1.41, 16.21]; p=0.006
Low	80	3 (3.8)	1.47	[0.34, 6.42]; p=0.69	4 (5.0)	2.62	[0.60, 11.41]; p=0.23
pCR	157	4 (2.5)	REF		3 (1.9)	REF	
gRDBN (n=459)							
High	81	31 (38.3)	15.02	[5.49, 41.09]; p<0.0001	19 (23.5)	12.28	[3.74, 40.26]; p<0.0001
Intermed	149	32 (21.5)	8.43	[3.05, 23.26]; p<0.0001	16 (10.7)	5.62	[1.67, 18.89]; p=0.003
Low	72	2 (2.8)	1.09	[0.20, 5.82]; p=1	2 (2.8)	1.45	[0.25, 8.51]; p=1
pCR	157	4 (2.5)	REF		3 (1.9)	REF	

Table 2; Reclassification of gRDBN categories

gRDBN		mRDBN Reclassification		
Classification	n	Low (%)	Intermed (%)	High (%)
Low	72	72 (100)	0 (0)	0 (0)
Intermed	149	8 (5.4)	140 (93.9)	1 (0.7)
High	81	0 (0)	24 (29.6)	57 (70.4)

Conclusions: Both mRDBN and gRDBN provide prognostic information; however, separation of categories is improved with mRDBN (Table 1). mRDBN reclassified 30% of the high risk-gRDBN patients into intermediate risk category with a recurrence rate of 20%, leaving the 'true' high risk subgroup with a revised recurrence rate of 50% (Table 2). RDBN index also identified a group of low risk patients who have prognosis similar to patients with pCR.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-15

Title: Mechanism of drug resistance in relation to sites of metastases: Meta-analyses of randomized controlled trials in advanced breast cancer

Niraula S and Ocana A. University of Manitoba and CancerCare Manitoba, Winnipeg, MB, Canada and 2Translational Research Unit, Albacete University Hospital, Albacete, Spain.

Body: Background: Breast cancer is heterogeneous at different levels: biologic subtypes, intratumoral areas, and sites of metastases. In clinical studies, metastatic sites are usually classified as visceral or non-visceral, but this has little influence in treatment decisions particularly in the absence of clinical urgency. Indeed, it is unclear if response to new treatments differs among sites of metastatic lesions.

Methods: Randomized controlled trials (RCTs) investigating 3 different anticancer strategies in metastatic breast cancer were identified:

- (1) Comparison of two endocrine strategies
- (2) Endocrine therapy and targeted therapy compared with endocrine therapy alone
- (3) New HER-2 targeted therapy compared with existing HER-2 targeted therapy

RCTs reporting hazard ratios (HR) for Progression Free Survival (PFS) and Overall Survival (OS) for sub-groups based on sites of metastases were weighted using generic inverse variance approach, and pooled in meta-analyses using Revman 5.3.

Subgroup difference was tested with Chi² and heterogeneity with I² statistics.

Results: A total of 11 RCTs (6,701 pts) qualified.

Summary of included studies

Study/Author	Comparisons	N(Visceral)	N(Non-Visceral)	HR for PFS(Visceral)	HR for PFS(Non-Visceral)
Bergh et al.	Endocrine Vs Endocrine	258	256	0.81[0.63,1.04]	1.10[0.86,1.41]
Chia et al.	"	395	298	0.88[0.74,1.05]	1.01[0.81,1.26]
Johnson et al.	"	281	193	0.93[0.73,1.18]	1.37[0.83,2.26]
Mehta et al.	"	348	195	0.79[0.63,0.99]	0.84[0.62,1.14]
BOLERO2	Targeted + Endocrine Vs Endocrine	406	318	0.45[0.35,0.58]	0.42[0.28,0.63]
PALOMA1	"	80	85	0.55[0.32,0.95]	0.29[0.09,0.93]
PALOMA2	"	324	342	0.63[0.47,0.84]	0.50[0.36,0.69]
PALOMA3	"	311	210	0.47[0.34,0.65]	0.55[0.45,0.67]
CLEOPATRA	New Vs Existing Anti-HER2	630	178	0.64[0.53,0.77]	0.83[0.58,1.19]
EMELIA	"	669	322	0.55[0.45,0.67]	0.96[0.71,1.30]
TH3RESA	"	452	150	0.56[0.44,0.71]	0.41[0.26,0.65]

There was a significant difference in PFS between women with visceral Vs non-visceral metastases when two endocrine strategies were compared, with benefits limited to women with visceral metastases [Pooled HR 0.85; 95% CI, 0.77-0.95 Vs 1.02(0.88-1.18) for non-visceral; p(difference) 0.05]. However, combination of an endocrine therapy and a targeted therapy was associated with better PFS compared to endocrine therapy alone for both groups [HR 0.51(0.43-0.60) for visceral Vs 0.45(0.36-0.56) for non-visceral; p(difference) 0.36]. Newer HER-2 targeted therapies were associated with significantly better PFS only in visceral but not in non-visceral metastases [HR 0.59 (0.52-0.66) Vs 0.71(0.44-1.13), p(difference) 0.45]. OS benefit with new HER-2 therapies was observed only for women with visceral metastases [HR 0.64 (0.56, 0.73) Vs 0.82 (0.57, 1.19), p(difference):0.20].

Conclusion: Targeted + endocrine therapy combination results in concordant, superior PFS suggesting similar mechanisms of

targetable endocrine resistance between metastatic sites. Discordant responses with endocrine strategy alone support use of targeted therapy, rather than change in endocrine agent at disease progression. New HER2 therapies display continued challenge of drug penetration to areas of limited vascularization (eg. soft tissue, bone) with no PFS or OS benefit in that group despite impressive benefits in overall population.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-16

Title: Identification of proliferation related drivers and their roles in precision medicine for breast cancers: A retrospective multidimensional comparative, integrated genomic, transcriptomic, and protein analysis

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Body: Background and Aim: The best test to guide the choice of systemic therapy for breast cancer (BC) has not yet been identified. We did this study to identify factors that drive proliferation features in BC and assess their association with clinical outcomes after systemic therapy.

Methods: We applied an artificial neural network-based integrative data mining approach to three cohorts of patients with untreated lymph node (LN)-negative BC (Wang et al; n=286, Desmedt et al; n=198 and Schmidt et al; n=200). The results were validated in four cohorts of BC patients (the Nottingham discovery cohort (n=171), Uppsala cohort (n=249), The Cancer Genome Atlas-Breast Cancer project [TCGA-BRCA; n= 970] and Molecular Taxonomy of Breast Cancer International Consortium [METABRIC cohort; n=1980]. Genes that featured prominently in our interactome map of proliferation have been chosen to take them forward to investigate their clinicopathological relevance of their gene copy number aberrations (CNAs), mRNA transcript expression, and protein expression and their associations with breast cancer-specific survival (BCSS), distant relapse-free survival (DRFS) and pathological complete response (pCR) in ten international cohorts of BC (n>12000 patients).

Findings: *ESR1*, *SPAG5*, *EGFR*, *BCL2*, and *FOXA1* were among the 39 common gene probes that were predictive across most proliferation features and datasets. In TCGA-BRCA cohort, *SPAG5* gene mutation, gain/amplification and loss at the Ch17q11.2 locus were detected in 43 (4.4%), 177 (18.2%) and 180 (18.8%) of 970 patients, respectively and 65 (31%) of 479 ER-positive/HER-positive patients showed gain/amplification of *SPAG5* gene. In multivariable analysis, high *SPAG5* transcript and *SPAG5* protein expression were associated with reduced BCSS compared with lower expression (METABRIC: HR 1.27, 95% CI 1.02–1.58, p=0.034; untreated LN-negative cohort: 2.34, 1.24–4.42, p=0.0090; and Nottingham-cohort: 1.73, 1.23–2.46, p=0.0020). In patients with ER-negative/HER2-negative or ER-positive/HER2-negative BC, high *SPAG5* transcript expression was associated with an increased pCR compared with low *SPAG5* transcript expression after receiving anthracycline neoadjuvant chemotherapy (AC-NeoACT) [(Multicentre phase 2 clinical trial cohort; n=136; OR 2.47, 95% CI 1.17–5.21, p=0.016) and (MD Anderson- taxane+AC-NeoACT cohort; n=287; OR 3.16, 95% CI 1.46–6.84, p=0.003); respectively]. In patients with ER-positive/HER2-negative BC who received taxane+AC-NeoACT followed by adjuvant tamoxifen (Adj-Tam) for 5 years (MD Anderson- taxane+AC-NeoACT cohort; n=287), high and low *SPAG5* transcript expression had similar DRFS (HR 1.40, 95% CI 0.76–2.58, p=0.282). Whereas in ER-positive/HER2-negative BC patients who received only adj-Tam (n=298), high *SPAG5* transcript expression was associated with reduced DRF at 5 years compared with lower expression (HR 1.98, 95% CI 1.19–3.27, p=0.008).

Interpretation: The transcript and protein products of *SPAG5* are independent prognostic and predictive biomarkers that might have clinical utility as biomarkers for combination cytotoxic chemotherapy sensitivity in ER-positive/HER-negative BC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-17

Title: Evaluation of a risk score based on biologic factors to enhance prognostic stratification by the American Joint Committee on Cancer (AJCC) Staging System

Mittendorf EA A, Vila J, Yi M, Chavez-MacGregor M, Chen RL L, Giordano SH H and Hunt KK K. University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: The 7th edition AJCC staging system provides prognostic information based on the anatomic extent of disease determined by the tumor size (T), lymph node status (N), and presence or absence of metastatic disease (M). Tumor biology, including grade, estrogen receptor (ER) status and HER2 status, which are known to have prognostic and predictive value, are not incorporated. This study was undertaken to evaluate a risk score that takes into account the tumor grade and other biomarkers that can be added to the current anatomic TNM staging system to improve stratification of patients with respect to disease specific survival (DSS) and overall survival (OS).

Methods: A prospectively maintained database was used to identify 3,327 patients with non-metastatic invasive breast cancer who underwent surgery as a first intervention from January 2007 through December 2013. Clinicopathologic data were recorded including: age, grade, ER status, HER2 status, and pathologic stage. Pathologic stage was determined according to the 7th edition of the AJCC staging guidelines. ER status was recorded as the percentage of cells staining positive by immunohistochemistry (IHC). Prior to 2010, tumors were classified as ER positive if there was >10% staining. A cut-off of 1% was used for patients treated after 2010, consistent with the change in American Society of Clinical Oncology (ASCO) guidelines. HER2 status was defined as positive if 3+ on immunohistochemistry or gene amplification was shown on fluorescence in situ hybridization. A risk score was calculated by assigning one point for each of the following tumor characteristics: ER-negative status, HER2 negative status and grade 3. Univariate survival analyses according to AJCC stage (I, IIA, IIB, IIIA and IIIC) and risk score (0-3) were performed for DSS and OS using the Kaplan Meier method.

Results: Median follow-up time was 5.0 years (range, 0.1 to 8.8). Five year DSS for the entire cohort was 97.9% (95% CI: 97.3%-98.4%). The distribution in risk score in the entire cohort was: risk score 0=81 (2.4%), 1=2289 (68.8%), 2=683 (20.5%), and 3= (8.3%). As shown in the table, for all AJCC stages, the 5-yr DSS and 5-yr OS varied according to risk score (p<.01).

Stage	Risk Score	n	5-yr DSS (%)	95% CI	5-yr OS (%)	95% CI
I (IA and IB)	0	36	100		97	80.4-99.6
	1	1173	99.4	98.7-99.7	96.7	95.4-97.6
	2	274	98.8	96.4-100.6	94.6	91.0-96.8
	3	119	96.6	91.1-98.7	93.8	87.5-97.0
IIA	0	31	100		96.8	79.2-99.5
	1	634	99.4	97.5-99.8	97.1	94.7-98.4
	2	236	97.5	93.2-99.1	94.1	88.7-97.0
	3	98	91.0	81.8-95.7	88.2	78.5-93.8
IIB	0	11	100		100	
	1	309	96.9	92.6-98.8	94.6	89.6-97.2
	2	107	92.9	83.6-97.1	89.3	80.1-94.4
	3	40	91.5	75.6-97.2	91.5	75.6-97.2
IIIA	0	3	100		100	
	1	134	98.3	88.2-99.8	91.5	82.6-96.0
	2	50	92.2	77.2-97.5	90.3	75.7-96.3
	3	7	68.6	21.3-91.2	68.6	21.3-91.2
IIIC	0	0				

	1	39	92.2	72.1-98.0	84.4	63.7-93.9
	2	16	80.8	51.4-93.4	80.8	51.4-93.4
	3	10	33.3	6.3-64.6	33.3	6.2-64.6

Conclusion: The current study demonstrates that incorporating the risk score with current AJCC staging significantly improves the ability to stratify breast cancer patients with respect to DSS and OS. We recommend that the risk score be incorporated into the forthcoming revision of the AJCC staging system.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-18

Title: Predictive factors of pathologic complete response to neoadjuvant chemotherapy in patients with Luminal HER2(-) local advanced breast cancer using the DMET microarray

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Body: Introduction

Patients with similar local advanced breast cancer (LABC) could respond different to neoadjuvant chemotherapy (NC). Luminal HER2(-) tumors have pathologic complete response (pCR) by 5-7%. Ki67, tumor grade and hormonal receptors, could be helpful but not enough to choice between NC vs. initial surgery. Response to drugs depends on enzymes involved in absorption, distribution, metabolism and elimination (ADME); the drug-metabolizing enzyme and transporter platform (DMET), could identify 1936 single nucleotide polymorphism (SNP) from 225 genes involved in ADME process.

Objective

To identify predictive factors to pCR in patients with Luminal HER2(-) LABC underwent NC, to distinguish wish patients could have a real benefit from NC.

Material and Methods

This is a prospective nested case control study (1:4) with LABC patients, pure invasive ductal carcinoma Luminal HER2(-) subtypes, and sequential NC (anthracyclines and taxanes). Cases were defined as patients with pCR (Residual Cancer Burden=0), and controls those without pCR. SNPs were evaluated from DNA of leucocytes using DMET microarrays by Affymetrix.

Bivariate analyses were used as appropriate. Microarrays were processed by the DMET analyzer console. OR and 95%CI were used to test association between pCR and variables with a non-conditional logistic regression analysis; p value ≤ 0.05 was considered statistically significant (two-sided).

Results

From 2005 to 2014, of 3762 treated patients, 287 women met inclusion criteria. We included 117 patients, 21 cases and 96 controls. Patients with pCR were 5 years younger than controls (45.4 vs 49.4), have more grade-3 tumors, (66.7 vs 22.3%), more Luminal B subtypes (90.5 vs 64.6%), lower expression of estrogen receptor (60 vs 80%) and higher Ki67 expression (65 vs 12%), all with $p \leq 0.05$. No differences in toxicity existed between groups.

In an initial screening procedure, 13 SNPs were identified, but only 4 SNPs (4 genes) are known to participate in ADME of antineoplastic agents. All pCR cases did not have the C/C allelic variant in the rs2072671 SNP (CDA gene); while in rs1883322 SNP (PPARD gene) all had T/T variant

SNPs identified in DMET microarray known to participate with antineoplastic agents

GENE	SNP	Allelic variant	pCR, n=21	No pCR, n=96	p
DPYD	rs17376848	T/T	13 (61.9%)	83 (86.4%)	0.007
		C/T	8 (38.1%)	11 (11.4%)	
CDA	rs2072671	A/A	6 (28.5%)	51 (53.1%)	0.013
		A/C	15 (71.4%)	36 (37.5%)	
		C/C	0	9 (9.3%)	
PPARD	rs1883322	T/T	21 (100%)	69 (71.8%)	0.022
		C/T	0	22 (22.9%)	
		C/C	0	5 (5.2%)	
GSTM3	rs7483	A/A	7 (33.3%)	20 (20.8%)	0.024

		A/G	13 (61.9%)	43 (44.7%)	
		G/G	1 (4.7%)	33 (34.3%)	

pCR= pathologic complete response

After logistic regression analysis, pCR predictive factors were the presence of grade-3 tumors OR=6.66 (95%CI=1.67-26.56), Ki67 \geq 14% OR=15.45 (95%CI=1.71–138.86), premenopausal status OR=6.540 (95%CI=1.50–28.38) and A/A or A/G allele in rs7483 SNP (GSTM3 gene) OR=19.16 (95%CI=1.97–185.93).

Conclusions

Genetic variability encoded in the DMET-chip, could be helpful as a predictive factor of pCR in patients with Luminal HER2(-) LABC, but only in combination with other factors such as grade-3 tumors, elevated Ki67, and premenopausal status.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-19

Title: Breast density change at 6 months is associated with change at 12 months as measured by fat-water decomposition MRI in women on tamoxifen

Ding J, Thompson PA A, Wertheim BC C, Roe DJ J, Marron MT T, Altbach MI I, Galons J-P, Wang F, Thomson CA A, Huang C and Stopeck A. Stony Brook University, Stony Brook, NY; Stony Brook Medicine, Stony Brook, NY and University of Arizona, Tucson, AZ.

Body: Objective: Tamoxifen (TAM) lowers breast cancer recurrence by 40-50% with evidence of individual variability in responsiveness. A $\geq 10\%$ decrease in mammography-determined breast density (BD) after 12–18 months of TAM use has been associated with clinical benefit. Early determination of changes in BD may offer a strategy to tailor hormone therapy in non-responders; for responders, it may encourage adherence. Fat-water decomposition MRI (FWD-MRI) is an accurate and fast (< 5 minutes) method for measuring BD without ionizing radiation or contrast agent. Here, we examined whether change in FWD-MRI-derived BD predicts decrease in BD at earlier time points than observable with a 12-month measure of BD.

Methods: The study population included a subset of 44 pre- and post-menopausal women receiving TAM for treatment of early-stage breast cancer or prevention who were enrolled in a randomized, placebo-controlled trial of diindolylmethane. Eligibility for this analysis included participants with FWD-MRI scans at baseline, 6 and 12 months. Median time on TAM at baseline was 13 months (IQR, 5–26 months). All MRI images were acquired on a 1.5T GE Signa NV-CV/i scanner. Automated breast segmentation was performed using MATLAB software and validated against manual ROI drawings. MRI-based BD was calculated as the ratio of breast voxels with <80% apparent fat fraction (Fra_{80}) over the entire breast, a measure previously shown by our group to be highly correlated with mammography-derived BD. For 40 participants, the unaffected, contralateral breast was analyzed. For 4 patients with two unaffected breasts, BD data from the left breast were analyzed. Change in BD was conservatively defined as > 2 times the test-retest variability of Fra_{80} (0.032). McNemar's test was used to test the association between change from baseline to 6 months and change from baseline to 12 months.

Results and Discussion: At 12 months, 15 (34%) participants had a decrease in BD, whereas 29 (66%) remained unchanged or increased. Of these 29, 28 also had no decrease at 6 months (specificity = 97%), and 9 of the 15 women who showed a decrease at 12 months had a decrease at 6 months (sensitivity = 60%; McNemar's test, $P = 0.06$). Conversely, for those women with a measured decrease in BD from baseline to 6 months, 9 of 10 had a measured decrease at 12 months. A study limitation is inclusion of participants on TAM for varying duration as the greatest change in BD likely would have occurred earlier. Ongoing efforts will focus on FWD-MRI for measures of change in BD in patients initiating TAM.

Conclusion: Use of the specified cut point would fail to detect a decrease in BD at 12 months in 40% of women. However, a decrease in BD from baseline to 6 months was highly associated with decrease from baseline to 12 months and in some women may be useful as an early biomarker of effect. Ongoing effort is needed to determine the impact of factors such as baseline BD, menopausal status, and time on TAM in misclassification of BD change using the 6-month measure.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-20

Title: Clinical utility of PgR with various cutpoints using 3 commercial assays relative to 15yr survival

Kornaga EN N, Paterson AHG HG, Feng X, Morris DG G, Magliocco AM M and Klimowicz AC C. Translational Laboratories, Tom Baker Cancer Centre, Alberta Health Services, Calgary, AB, Canada; University of Calgary, Calgary, AB, Canada; BC Cancer Agency, Vancouver Island Cancer Centre, University of British Columbia, Victoria, BC, Canada; Anatomic Pathology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL and Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT.

Body:

Introduction:

Hormone receptors ER and PgR are routinely assessed by pathologists using immunohistochemical (IHC) assays to guide treatment decisions. Patients who are hormone receptor positive are offered hormonal therapy, such as tamoxifen, which improves survival. Although both ER and PgR are evaluated, ER is primarily utilized for patient management as the clinical utility of PgR has not been clearly established according to CAP/ASCO guidelines. Notably, a meta-analysis by the Early Breast Cancer Trialists' Collaborative Group reported that PgR status was not significantly predictive of response to adjuvant tamoxifen, suggesting that PgR may not have a role in breast cancer management. More recently, the level of PgR expression has been hypothesized to be important in predicting response to endocrine therapy, where high PgR levels are more indicative of estrogen-dependent tumors, and thus more sensitive to hormonal treatment.

In this study, we evaluate PgR expression using the current cut-point (Allred>2), as well as an optimized cut-point (Allred>5 to identify PgR high tumors), with regards to 15yr disease-free survival (DFS) and disease-specific overall survival (DSOS) using three commercially available ready-to-use (RTU) IHC assays from Dako, Leica and Ventana in an ER+ cohort.

Methods:

The Calgary tamoxifen breast cancer cohort (Calgary cohort) is a TMA series that includes 532 patients diagnosed with primary breast cancer (1985-2000) who received tamoxifen treatment regardless of hormone receptor status. All RTU assays followed vendor recommended protocols. Specific details regarding the cohort and IHC assays have been previously described (Kornaga et al. Mod Path 2016). All analyses were performed using Stata 12, and multivariate models were adjusted for age, grade, size, lymph node and HER2 status. ER status was defined by the corresponding vendor specific IHC assay.

Results:

Multivariate models looking at DFS are presented in Table 1. None of the assays were significant when the clinical cut-point was used; however, when the optimized cut-point was investigated, all assays found high expression of PgR was significantly associated with improved DFS. Table 2 presents the multivariate models looking at DSOS. Similarly, PgR was not found to be associated with improved DSOS using the current cut-point. When the optimized cut-point was examined, Dako and Leica assays were significantly associated with improved DSOS: The Ventana assay did not reach significance.

Table 1

	PgR Cutpoint	15YR DFS		
		HR	95% CI	p-value
Dako	>2	0.83	0.40-1.73	0.624
Dako	>5	0.56	0.35-0.91	0.020
Leica	>2	1.13	0.45-2.83	0.792
Leica	>5	0.52	0.30-0.89	0.017
Ventana	>2	0.73	0.32-1.67	0.460
Ventana	>5	0.57	0.34-0.97	0.037

Table 2

	PgR	15YR DSOS		
	Cutpoint	HR	95% CI	p-value
Dako	>2	0.74	0.33-1.65	0.464
Dako	>5	0.55	0.32-0.95	0.031
Leica	>2	0.88	0.35-2.22	0.780
Leica	>5	0.44	0.24-0.79	0.006
Ventana	>2	0.59	0.25-1.37	0.218
Ventana	>5	0.63	0.35-1.15	0.134

Conclusions:

High PgR expression (Allred>5) is associated with improved 15yr DFS and DSOS in a tamoxifen-treated cohort, where PgR positivity defined using current guidelines is not associated with improved DFS or DSOS. Additionally, differences were noted between the vendor RTU assays with regards to DSOS.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-21

Title: Prognostic impact of metastatic pattern in stage IV breast cancer at initial diagnosis

Leone BA A, Vallejo CT T, Romero AO O, Machiavelli MR R, Perez JE E, Leone J and Leone JP P. Grupo Oncológico Cooperativo del Sur (GOCS), Neuquén, Neuquén, Argentina; Grupo Oncológico Cooperativo del Sur (GOCS), Bahía Blanca, Buenos Aires, Argentina and University of Iowa Holden Comprehensive Cancer Center, Iowa City, IA.

Body: Background: Stage IV breast cancer at initial diagnosis (BCID) can be recognized in approximately 5% of all breast neoplasms. Clinical outcomes of these patients (pts) are highly variable and depend on tumor biology and pt characteristics. The prognostic influence of metastatic pattern (MP) at initial presentation and factors associated with specific organ involvement have been understudied. The primary aim of this study was to analyze the influence of MP compared with other biologic and clinical factors in the survival of pts with stage IV BCID. The secondary aim was to evaluate factors associated with specific sites of metastatic spread.

Methods: We evaluated women with microscopically confirmed stage IV BCID with known metastatic sites, reported to the Surveillance, Epidemiology and End Results (SEER) 18 registries program from 2010 to 2013. Pts with other primary tumor either before or after breast cancer were excluded. MP was categorized as bone only, visceral (lung, liver or brain), bone and visceral and other. Pt characteristics were compared between MP. Univariate and multivariate analyses determined the effects of each variable on overall survival (OS). Logistic regression examined factors associated with specific sites of metastases.

Results: We included 9143 pts. Median age was 61 years (range 19-102). Median OS for the entire cohort was 28 months (95% CI 27-29 months). At diagnosis, bone only metastases represented 37.5% of pts, visceral 21.9%, bone and visceral 28.8% and other 11.9%. Median OS by MP was: bone only 38 months, visceral 21 months, bone and visceral 19 months and other 33 months ($p<0.0001$). Bone was the most common site of metastases (66.2%), followed by lung (30.5%), liver (26.2%) and brain (7.4%). Pts with visceral metastases were more often black race, had higher grade, less likely to be lobular histology and more likely to be triple negative (TN) (all $p<0.0001$). Univariate analysis showed that older age, black race, grade 3/4 tumors, TN pts, bone and visceral MP, higher number of metastatic sites and unmarried pts had worse prognosis (all $p<0.0001$). In multivariate analysis, older age (HR 1.9; $p<0.001$), black race (HR 1.17; $p=0.002$), grade 3/4 tumors (HR 1.6; $p<0.001$), TN subtype (HR 2.24; $p<0.001$), bone and visceral MP (HR 2.07, $p<0.001$) and unmarried pts (HR 1.25; $p<0.001$) had significantly shorter OS. In adjusted logistic regression, as compared with ER/PR+/HER2- tumors, TN and ER/PR-/HER2+ had higher odds of brain, liver, lung and other metastases (all $p<0.05$). ER/PR+/HER2+ had higher odds of liver metastases ($p<0.001$). All three subtypes had lower odds of bone metastases ($p<0.001$).

Conclusions: To our knowledge, this is the largest study of MP in stage IV BCID. There were substantial differences in prognosis according to MP, bone only was the most common MP and had the best OS, whereas bone and visceral MP had the worst prognosis. We observed significant differences in pt characteristics according to MP. Independent predictors of OS included age at diagnosis, race, marital status, tumor grade, tumor subtype and MP. There was a clear influence of tumor subtype among other factors on specific sites of metastases. Our study identified several prognostic factors that could guide therapy selection in treatment naïve pts.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-22

Title: Detecting high mutational load ER+ breast cancer patients through Foundation One cancer gene panel mutations

Raska P, Abraham J and Budd T. Taussig Cancer Institute, Cleveland Clinic Foundation, Cleveland, OH.

Body: Background:

Cancer gene panels such as Foundation One are widely used clinically for aiding with cancer treatment decision-making since single point mutations in important genes and gene pathways in the tumor can point to tumor susceptibility that can be targeted with specific drugs. Tumor mutational load has more recently been proposed as a useful prognostic factor and indicator of clinical benefit in treatment with PD-1 and CTLA-4 blockade therapy. Breast cancer estrogen receptor positive patients (ER+) in particular appear to have a subset of high mutational load patients that could benefit if identified. However whole exome sequencing needed to directly measure mutational load is not yet widely available as a clinical tool. In this study we evaluate the predictive value of Foundation One cancer gene panel mutations for estimating tumor genome-wide mutational load and its use in identifying a clinically meaningful subset of breast cancer patients.

Methods:

The Cancer Genome Atlas breast cancer sequencing data on 569 ER+ patients was used to establish mutational load distribution. ER+ patients were divided into low mutational load and high mutational load groups according to 3 criteria: mean mutational load, the point of inflection in the mutational load distribution and the mutational load that optimally separates groups in terms of survival. Foundation One (FO) mutational load was then calculated as the number of mutations present within the 314 genes queried by the panel. FO mutational load was used to predict whether patients fell into the low or high mutational load groups found through analysis of the full exome data. Receiver Operating Characteristic (ROC) curves were constructed and optimal values for specificity and sensitivity of the FO mutational load classification were found.

Results:

Mean mutational load for ER+ patients was found to be 57 mutations, the point of inflection of the mutational load distribution was established at 100 mutations, and the number of mutations that best separated groups in terms of survival was 160, (HR = 6.6, p-value=0.004). FO mutational load was found to be a good predictor for the low and high classifications established by all three criteria, with areas under the curve of 0.74, 0.91 and 0.945 respectively. The optimal predictive value of the FO mutational load classification was found at 5 mutations as the cut-off, with 94.2% specificity and 88% sensitivity for predicting groups defined by survival and 95% specificity and 71% sensitivity for those defined by the mean.

Conclusion:

The Foundation One cancer gene panel can be used to effectively identify a clinically meaningful subgroup of ER+ patients with high mutational load. These patients may benefit from targeted treatments such as PD-1 inhibitors being offered through clinical trials. The compromise in sensitivity that results from the reduction in number of genes queried by a panel means an important proportion of patients with high mutational load will be missed but this still translates to a large improvement in the identification of these patients given the wide availability of gene panels in the clinic. Basic and clinical follow-up studies need to take place to clinically validate the high mutational load ER+ patient subgroup.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-23

Title: SET_{ER/PR} - A robust 18-gene predictor of sensitivity to endocrine therapy in metastatic breast cancer

Sinn BV V, Tsai T-H, Lau R, Fu C, Gould R, Murthy R, King TA A, Hatzis C, Kwiatkowski DN N, Valero V and Symmans WF F. The University of Texas MD Anderson Cancer Center, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX; Brigham and Women's / Dana Farber Cancer Center, Boston, MA; Section of Breast Medical Oncology, Yale School of Medicine, Yale Cancer Center, New Haven, CT; The University of Texas MD Anderson Cancer Center, Houston, TX and Charité Universitätsmedizin Berlin, Berlin, Germany.

Body: Rationale: A robust index for gene expression related to activity of estrogen (ESR1) and progesterone (PGR) receptors could predict sensitivity to endocrine therapy in metastatic breast cancer.

Methods: Transcripts correlated with ESR1 and PGR expression in 389 hormone receptor-positive breast cancer samples (Affymetrix U133A microarrays) were ranked for reliability according to their pre-analytical (intratumoral heterogeneity, biopsy type) and analytical reproducibility. Eighteen target and ten reference genes were selected and summarized as the SET_{ER/PR} index. The SET_{ER/PR} index was evaluated in a different set of 140 biopsies from distant metastases of hormone receptor-positive and HER2-negative (HR+/HER2-) breast cancer, and in additional pre-analytical and analytical sample cohorts. Thereafter, SET_{ER/PR} was translated to a customized format for application to formalin-fixed and paraffin-embedded (FFPE) sections.

Results: Higher SET_{ER/PR} in a metastasis was associated with longer progression-free survival (PFS, 9 vs. 2 months) and overall survival (OS, 50 vs. 19 months) following endocrine therapy in the cohort with metastatic breast cancer (MBC) and relapsed disease (n=79), so a cut point was defined in that cohort. SET_{ER/PR} was also significantly associated with PFS after adjusting for PR status of the metastasis, presence of visceral metastases, number of previous relapse events, and clinical history of previous sensitivity to endocrine therapy (HR 0.485, 95%CI 0.265 – 0.889, p = 0.019). Technically, SET_{ER/PR} was highly reproducible under different pre-analytical and analytical conditions, including host organ contamination. The translated SET_{ER/PR} assay used a single 10 µM FFPE tissue section, did not require RNA purification, and represented the microarray results from matched fresh samples with excellent agreement (correlation = 0.980, n = 31).

Conclusion: The SET_{ER/PR} index is a new biomarker to predict PFS and OS for patients with HR+/HER2- MBC who receive endocrine therapy. The assay is applicable to FFPE tissue sections from small biopsies of metastases. Additional independent (blinded) validation studies will be necessary to confirm these results.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-24

Title: Vitamin D level impacts odds of pathologic complete response following neoadjuvant therapy in operable breast cancer

Thomas A, Thezenas S, Mott SL L, Raman R, Viala M, Pouderoux S, Schroeder MC C, Lamy P-J and Jacot W. University of Iowa, Iowa City, IA and Institut Régional du Cancer de Montpellier (ICM), Montpellier, Languedoc, France.

Body: Introduction:

Pathologic complete response (pCR) following neoadjuvant systemic chemotherapy (NAC) serves as a measure of tumor responsiveness and is a recognized surrogate for improved long-term outcomes. Some, but not all, studies have found a positive association between vitamin D (VD) and disease-free survival. We investigated if VD at diagnosis or during chemotherapy impacts pCR following neoadjuvant chemotherapy for operable breast cancer.

Methods:

Patients from Iowa were eligible if they were enrolled in one of two Iowa registries and had serum from before or during NAC tested for VD. French patients enrolled in a previous study of the impact of NAC on vitamin D and bone metabolism were considered for this study. VD deficiency was defined as <20 ng/ml. pCR was defined as no residual invasive disease in breast and lymph nodes. Firth-penalized logistic regression multivariable model was used.

Results:

The final cohort included 144 women. 84.7% of VD levels were obtained before initiation of chemotherapy. There was no difference between the French and Iowa cohorts with regard to age ($p=0.20$), clinical stage ($p=0.22$), disease receptor status (HER2+ [Hormone receptor (HR)+ or HR-], HR+/HER2-, and Triple Negative) ($p=0.32$) and rate of pCR ($p=0.34$). French women had lower body mass index (mean 24.8 vs 28.8, $p<0.01$), lower VD levels (mean 21.5 vs 27.5, $p<0.01$) and underwent lumpectomy instead of mastectomy more frequently (75.3% vs 47.8%, $p<0.01$) than Iowa women. Only pCR differed between the VD sufficient and deficient groups (Table 1). In multivariate analysis, after adjusting for the effects of cohort, clinical stage, and disease type by receptor status, VD deficiency put a woman at 2.68 times increased odds of not attaining a pCR (95%CI: 1.12-6.41, $p=0.03$) (Table 2). This variable remained significant with VD deficiency defined as <30 ng/ml and when considering this variable continuously.

Conclusion:

In this retrospective cohort, VD level before or during NAC was associated with pCR. Prospective trials could elucidate if maintaining VD levels during NAC, a highly modifiable variable, can be utilized to improve cancer outcomes in addition to benefiting other established health outcomes.

Table 1: VD Deficient and Sufficient Groups

		Vitamin D (ng/ml)		p
		Deficient (< 20)	Sufficient (≥ 20)	
N		53	91	
BMI	Underweight-Normal	27 (50.9)	47 (51.6)	0.93
	Overweight-Obese	26 (49.1)	44 (48.4)	
VD Level	Before Chemotherapy	45 (84.9)	77 (84.6)	0.96
	During Chemotherapy	8 (15.1)	14 (15.4)	
Grade	G1-2	24 (47.1)	41 (46.1)	0.91
	G3	27 (52.9)	48 (53.9)	
Clinical Stage	I-II	37 (69.8)	61 (67)	0.73
	III	16 (30.2)	30 (33)	
Receptor Status	HER2+ (HR+ or HR-)	12 (22.6)	29 (32.2)	0.20

	HR+/HER2-	30 (56.6)	37 (41.1)	
	Triple Negative	11 (20.8)	24 (26.7)	
Surgery	Breast Conserving	32 (60.4)	58 (63.7)	0.69
	Mastectomy	21 (39.6)	33 (36.3)	
pCR	No	43 (81.1)	53 (58.2)	<.01
	Yes	10 (18.9)	38 (41.8)	
Age	Mean (SD)	48 (10.9)	51 (10.3)	0.15

Table 2: Odds of Not Attaining a pCR: Multivariable Results

		N	Odds Ratio	95% CI		p-value
Cohort	Iowa	66	1.17	0.52	2.63	0.71
	France	77	Ref			
Vitamin D (<20)	Deficient	53	2.68	1.12	6.41	0.03
	Sufficient	90	Ref			
Clinical Stage	I-II	97	Ref			
	III	46	3.25	1.30	8.11	0.01
Receptor Status	HER2+ (HR+ or HR-)	41	1.06	0.40	2.81	0.91
	HR+/HER2-	67	5.50	2.04	14.85	<0.01
	Triple Negative	35	Ref			

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-25

Title: Circulating tumor cell clusters-associated gene plakoglobin is a novel prognostic predictor in patients with breast cancer

Goto W, Kashiwagi S, Asano Y, Takada K, Takashima T, Morisaki T, Noda S, Onoda N, Ohsawa M, Hirakawa K and Ohira M. Osaka City University Graduate School of Medicine, Osaka, Japan and Osaka City University Graduate School of Medicine, Osaka, Japan.

Body: Background: Accumulating evidence shows that circulating tumor cells (CTC) are linked to metastatic relapse and are regarded as a prognostic marker for human cancer. It was reported that CTC clusters (CTCc) have more metastatic potential than single CTC. Lately, studies also show that the high expression of plakoglobin, a cell adhesion protein, within the primary tumor are positively associated with CTCc in breast cancer patients. In addition, it is thought that insufficient expression of plakoglobin could promote epithelial-mesenchymal transition (EMT). In this study, we investigated the correlation between plakoglobin expression and survival of breast cancer.

Materials and Methods: A total of 126 patients with resectable early-stage breast cancer were treated with neoadjuvant chemotherapy (NAC). All patients received a standardized protocol of NAC consisting of four courses of FEC100 (500 mg/m² fluorouracil, 100 mg/m² epirubicin, and 500 mg/m² cyclophosphamide) every 3 weeks, followed by 12 courses of 80 mg/m² paclitaxel administered weekly. The expression of plakoglobin were identified by immunohistochemical staining properties in cell membrane. Staining with plakoglobin (clone 4C12) was scored according to the percentage of cells that stained positively: low, 0-25%; medium, 26-75%; high, >75%. We investigated the correlation between the plakoglobin expression in primary tumor specimen and clinical outcomes including overall-survival (OS), disease-free-survival (DFS), distant-metastasis-free-survival (DMFS), the efficacy of NAC. And we examined the relation between the expression of plakoglobin and E-cadherin, EMT marker.

Results: The patient with high plakoglobin expression had significantly worse OS ($p=0.021$, log-rank) and DFS ($p=0.015$, log-rank), DMFS ($p=0.040$, log-rank). And the plakoglobin expression had no correlation with pathological complete response (pCR) rate ($p=0.596$). Also, there was not a statistically significant relationship between the plakoglobin expression and other clinicopathological parameters including tumor size ($p=0.708$), lymph node status ($p=0.479$), subtype ($p=0.413$), nuclear grade ($p=0.642$), Ki67 ($p=0.202$), tumor infiltrating lymphocytes ($p=0.828$). On univariate analysis with respect to distal metastasis, high plakoglobin expression showed worse prognosis than low plakoglobin expression ($p=0.013$, hazard ratio=4.221). And multivariate analysis found the same result ($p=0.015$, hazard ratio=4.070). In addition, there was a significant relationship between the expression of plakoglobin and E-cadherin ($p=0.023$).

Conclusions: Plakoglobin expression is an independent prognostic factor in the patients with breast cancer; particularly for DMFS, and this mechanism related to EMT.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-26

Title: Effect of race on triple negative breast cancer outcomes

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Body: Background: African American (AA) women and patients with Triple Negative Breast Cancer (TNBC) have poor outcomes. It is unclear whether these worse outcomes in AA women are due to a higher frequency of TNBC in AA women. The purpose of our study was to determine difference in outcomes between AA and Caucasian (CA) female with TNBC.

Methods: A retrospective study of women diagnosed with ER, PR and HER2 (1+) negative breast cancer between January 1/2001 and December 31/2013 at Vidant Medical Center (East Carolina University) in Greenville, North Carolina was undertaken. Patient demographics and tumor characteristics were analyzed to determine impact on overall survival.

Results: In our institution, 4,434 women were diagnosed with breast cancer in this study period, of which 378 had TNBC. Our TNBC population was predominantly AA (53%). AA patients were younger (54.1 years vs 58.7 years, $p=0.005$), less often postmenopausal (62.6% vs 74.5%, $p=0.013$), more likely to have Medicaid (24.3% vs 7.9%, $p < 0.001$), and to have received chemotherapy (80% vs 69%, $p=0.017$) compared to CA women. There was no difference in stage at diagnosis between AA and CA patients ($p=0.12$). By univariate analysis, improved survival in TNBC was associated with non-Medicaid status ($p=0.01$), early stage at diagnosis ($p=0.001$), N stage (<0.001), and tumor grade ($p < 0.001$). A Cox regression analysis demonstrated that only insurance status ($p=0.007$) and N Stage ($p=0.01$) predicted outcomes in TNBC. Survival in TNBC was not affected by race ($p=0.7$).

Conclusions: Poorer outcomes in AA women cannot be attributed to the higher frequency of TNBC. Improved access to healthcare, with broader insurance coverage, may help minimize disparities in outcome by diagnosing TNBC at an earlier stage.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-27

Title: The EA2clin test significantly predicts response and survival in both pre and post-menopausal women with ER-positive breast cancers

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Body: Background

Identifying breast cancer (BC) patients likely to recur on endocrine therapy (ET) is a challenge. Several methods and tests based on clinical parameters and multi-gene or protein classifiers have been shown to predict those likely to recur. Tests that incorporate baseline and on-treatment markers are likely to be more accurate than tests based on baseline characteristics alone. 4 genes were identified by microarray that predicted for to ET: 2 at diagnosis and 2 at 14 days. The EndoAdjuvant2 (EA2) test uses 2 of these genes: IL6ST at diagnosis and on-treatment MCM4 at transcript level or by graded immunohistochemistry (IHC). The aim of this study was to compare EA2 with currently used clinical parameters in 4 different cohorts of pre and postmenopausal women.

Patients

The cohorts are (1) 73 post-menopausal women (PMW) with ER+ BC treated with neoadjuvant letrozole (L) then surgery, (2) 39 PMW with ER+ BC treated with neoadjuvant anastrozole (A) then surgery, (3) 108 PMW who received 2-weeks of A or L prior to surgery (4) 25 preMW with ER+ BC who received one 750mg dose of fulvestrant prior to surgery. All had adjuvant ET and 5-10 years follow up. Neoadjuvant response was assessed by periodic 3D ultrasound.

Results

The 4 gene assay had 96% (training; cohort 1) and 93% (validation; cohort 2) accuracy of response prediction to neoadjuvant L or A. In cohort 1, clinical parameters were available. On univariate regression analysis intrinsic subtype (luminal A/B; defined using PAM50) ($P=0.002$), histological grade ($P=0.033$) and HER2 status ($P=0.001$) significantly predicted clinical response. EA2 out-performed all these in both univariate ($P<0.0001$) and multivariate regression analysis ($P<0.0001$). The final model only retained EA2 as significant. Node status, tumour size and PR expression were not associated with response to endocrine therapy.

EA2 predicted recurrence free in cohorts 1 and 3 combined: RFS ($P=0.0004$, HR=17.63 95%CI: 4.95-17.6), BCSS ($P=0.0007$, HR=16.60: 3.36-45.7). The Nottingham Prognostic Index (NPI) also predicted RFS ($P=0.0002$) and BCSS ($P=0.0017$) in univariate analysis but in the Cox analysis NPI was not found to be significant, although in the low risk group there were only 1/46 events compared to 19/62 in the moderate/high risk group. Histological grade ($P<0.0001$), Ki67 (%) ($P=0.02$) and tumour size ($P=0.007$) were significant on univariate analysis. In the Cox regression analysis, only EA2 ($P=0.012$) and histological grade ($P=0.016$) were significant predictors of RFS and BCSS. Using EA2 and NPI together a high and low risk subgroup could be identified. In the Cox regression analysis, combined EA2 plus NPI (EA2clin) outperformed both NPI and EA2 alone. EA2clin was validated in cohort 4 and predicted RFS ($P=0.002$, HR=8.43 95%CI: 2.18-32.53) and BCSS ($P=0.008$, HR=10.95 95%CI: 1.48-64.9).

Conclusions

- EA2 predicts clinical response, RFS and BCSS
- EA2clin combines NPI and EA2, outperforms either alone and predicts outcome in a new validation cohort of preMW treated with Fulvestrant
- EA2clin works in preM and PM women regardless of ET.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-28

Title: Prognostic impact of the inclusion of uPA/PAI-1 in ER+/Her2- pN0 early breast cancer adjuvant treatments decision-making

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Body: Purpose: Intermediate-risk early breast cancer (EBC) is a heterogeneous group in which adjuvant chemotherapy decision prove to be difficult. Clinical and pathological criteria might be insufficient to determine the best therapeutic options for patients, and validated biomarkers are urgently needed to help decision making, such as the LOE-I uPA/PAI-1 biomarker. The aim of this study is to evaluate the clinical outcome of a large, unselected, ER+/HER2- pN0 EBC cohort of patients in whom the routine clinical decision process included a prospective uPA/PAI-1 determination.

Methods: This monocentric retrospective study included 520 ER+/HER2- pN0/M0 EBC patients who had curative surgery in our center between 2006 and 2011. Adjuvant therapeutic strategy was decided based on clinico-pathological data, altogether with a routine prospective determination of uPA/PAI-1 tumor levels. We evaluated the correlation between uPA/PAI-1 levels, classical clinico-pathological variables and the patient's outcome (relapse free survival, RFS; overall survival, OS).

Results: Median age was 54 years (range 27-85). 75% of tumors were classified T1, 25% T2 and above. We found 80.8% of ductal carcinomas, 12.3% of lobular carcinomas and 6.9% of other histological types. SBR grade 1-2 represented 82.5% of our cohort versus 17.5% of grade 3. Mitotic count was <10 in 63.5% of cases, and ≥ 10 in 36.5%. Peri-tumoral invasion (PVI) was seen in 17% of tumors. Progesterone receptors (PR) were positive in 79.6% of cases. Median follow-up was 5.4 years. 5 and 10 years RFS were respectively 95% and 89% (n=33 local or metastatic relapse). 5 year OS was 96.3%. We found 40% of low uPA/PAI-1 levels, and 60% of high uPA and/or PAI-1 levels. By using uPA/PAI-1 levels in our adjuvant treatment decision-making, 75% of patients with low uPA/PAI-1 levels did not received chemotherapy and 60% of patients with high uPA and/or PAI-1 levels received chemotherapy. 98.3% of our patients received endocrine therapy as adjuvant treatment for at least 5 years. No statistical significant correlation was found between uPA/PAI-1 levels and RFS ($p=0.3$) or OS ($p=0.28$). In univariate analysis, tumor size ($p=0.048$; $p=0.0694$), histological grade ($p=0.007$; $p=0.0142$), PR status ($p=0.001$; $p=0.0002$) and mitotic count ($p=0.0001$; $p=0.0001$) were statistically correlated with RFS and OS, respectively. Using cox regression model, PR status ($p<0.003$) and mitotic count ($p<0.001$) appeared to be strongly correlated with RFS. No changes in significant variables were seen when the analyses were restricted to the grade 2 subgroup (n=339).

Conclusion: The individualization of patients' treatment using uPA/PAI-1 tumor levels allows the reversion of the well-known poor prognostic impact of high uPA/PAI-1 levels and strongly support the use of this LOE-I biomarker in clinical practice. PR status and mitotic activity remains independent major prognostic factors in optimally treated patients. The evaluation of the additional impact of multigene signature in this setting needs to be performed. Longer 10-years follow-up needs to be done in this ER+/HER2- subgroup, particularly to evaluate the risk of late relapse after endocrine therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-29

Title: Clinicopathological characteristics and survival outcomes in invasive papillary carcinoma of the breast: A population-based study

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Body: Background

Invasive papillary carcinoma (IPC) is defined as having papillary architecture in >90% of the invasive component. The overall incidence of IPC is low, accounting for less than 1-2% of all newly diagnosed cases of invasive breast cancer. Limited data are available that contribute to a comprehensive summarization of the clinicopathological characteristics and prognostic factors that are associated with IPC. We aimed to determine the clinicopathological characteristics and prognostic factors of IPC in a large population and help physicians to acquire a better understanding of the disease and make better informed therapeutic decisions.

Methods

We identified 233,171 female patients in the Surveillance, Epidemiology, and End Results (SEER) database who had IPC (n = 524) or infiltrating ductal carcinoma (IDC) (n = 232,647). The demographics and tumour and treatment characteristics of IPC were compared to those of IDC. A Cox proportional hazards model was used to investigate the effects of baseline characteristics on disease-specific survival (DSS). We performed a 1:1 (IPC: IDC) matched case-control analysis using the propensity score-matching method. A forest plot of hazard ratios (HRs) that was used to illustrate the exploratory subgroup analyses.

Results

Generally, IPCs occurred in older women (≥ 50 years old) and presented with smaller sizes, lower grades, higher rates of oestrogen receptor (ER) and progesterone receptor (PR) positivity, and reduced lymph node (LN) involvement and were less likely to be treated with mastectomy than patients with IDC. The five-year DSS rates were significantly better in IPC than in IDC (97.5% vs. 93%, respectively; $P < 0.001$). In the multivariate analysis, patients with IPC showed a DSS that was similar to that of IDC (HR = 0.556, 95% confidence interval 0.289–1.070, $P = 0.079$). No significant difference was observed in DSS between matched IPC and IDC groups ($P = 0.085$). Subgroup analyses suggested that differences in outcomes may be partially explained by differences in tumour grade, LN status, and ER and PR status between the 2 groups. Most IPCs are ER-positive tumours. When the analysis was limited to 178,755 ER-positive IPC and IDC patients (457 IPCs and 178,298 IDCs), similar results were observed.

Conclusions

This study is currently the largest analysis of IPC. We investigated a large cohort of patients with IPC and found that this rare tumour type presents unique clinicopathological characteristics and is associated with a higher rate of breast-conserving surgery and favourable prognoses than are observed in the overall IDC population. However, this advantage was diminished after we adjusted for demographic and clinicopathological factors. Therefore, patients diagnosed with this rare variant should be made aware that its biological features are not as favourable as once thought. Therapeutic decisions should not be made based solely on this rare entity and evidence-based treatment guidelines should be strictly followed. Improving our understanding of the clinical and biological features of IPC may lead to more individualized and tailored therapies for breast cancer patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-30

Title: Factors influencing survival among patients with HER2-positive metastatic breast cancer treated with Trastuzumab

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Body: Background: We have limited capability to predict survival among patients treated for metastatic HER2+ breast cancer. Individual patient survival varies and further research is warranted to identify significant prognostic and predictive factors influencing overall survival (OS).

Methods: We identified HER2+ metastatic breast cancer patients receiving trastuzumab (T) at the Sunnybrook Odette Cancer Centre (SOCC) from 1999-2013 through a Cancer Care Ontario Registry (n=256) and selected patients with pathology also available at SOCC (n=154). A retrospective chart review was completed documenting clinical, pathologic, laboratory and survival outcomes. OS was defined as date of 1st T therapy to death. The Kaplan-Meier method was used to estimate time-to-event outcomes. Cox proportional hazards regression models and log-rank tests were used to identify prognostic factors for overall survival (OS). Logarithmic transformations were performed for statistical purposes. Multivariable models were constructed including known prognostic factors: 1) number of visceral metastatic sites and 2) CNS metastasis. After adjusting for these two factors, stepwise selection was used to create an optimal model for additional factors. Analyses were two-sided and statistical significance was defined at the p=0.05 level.

Results: Cohort characteristics: mean age was 55 (SD: 13 years), ≥2 sites of visceral metastasis: 45%, CNS metastasis: 7%, ER positive: 53%. Median OS for the cohort was 24 months (95% CI: 21-33). Clinical factors recorded at metastatic presentation such as the presence of a visceral metastasis, having multiple sites of visceral metastasis and CNS metastasis were prognostic for overall survival in univariate models (p<0.05). ER/PR status was not of significance (p>0.05). Laboratory measures such as the neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) and alkaline phosphatase (ALP) were of significance in univariate models (p<0.05). The multivariable model identified older age (HR=1.18 / decade, 95% CI=1.02-1.37, p=0.030), higher PLR (HR=1.75 / log-unit, 95% CI=1.25-2.46, p=0.001), increased ALP (HR=1.87 / log-unit, 95% CI=1.41-2.49, p<0.001) and ER positivity (HR=0.63, 95% CI=0.42-0.96, p=0.032), as significant prognostic factors in addition to the presence of CNS metastasis (HR=3.19, 95% CI=1.59-6.38, p=0.001) and two or more metastatic sites (HR=2.10, 95% CI=1.19-3.70, p=0.010).

Conclusion: Our results have identified a number of prognostic factors influencing survival among patient with HER2+ breast cancer treated with T. Age, ALP, PLR and ER status were identified as significant prognostic factors after adjusting for presence of CNS metastasis and number of metastatic sites. Further study of PLR as a prognostic and predictive factor is warranted.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-31

Title: Study of oncotype DX® recurrent score in bilateral synchronous primary invasive breast cancer

Chadha J, Goel A, Wallach J, Shao T, Klein P and Malamud S. Mount Sinai West, New York, NY and Mount Sinai Health System, New York, NY.

Body: Background: The 21-gene Oncotype Dx® Recurrence Score (RS) is routinely used to assess the risk of distant recurrence and benefit for adding chemotherapy in the treatment of ER positive HER2 negative early stage breast cancer. Among patients diagnosed with bilateral primary breast cancer there is limited information on the utilization of RS for each primary cancer. In this study, we evaluate the concordance of RS in bilateral breast cancer, and the value of testing both primary cancers in patients diagnosed with synchronous bilateral breast cancer

Materials & Methods: This is an IRB approved retrospective study. From 2007 to present, we identified 158 patients with synchronous (within 6 months) bilateral primary breast cancer in our Institutional databases. In this dataset, there were 36 patients who had early stage ER positive, and Her-2 negative bilateral invasive cancer, and for whom the 21-gene Oncotype Dx® RS test was ordered. We excluded patients with bilateral invasive cancer who did not have Oncotype Dx® RS test performed (n=20), patients with bilateral in-situ cancers (n=15), patients with unilateral invasive with contralateral in-situ cancer (n= 64), and patients with locally advanced stage of one or both primary invasive cancers (n=23). In this study, RS score was scored low risk < 18, intermediate 18-30, and high risk >31. The RS for bilateral primary cancers was noted as concordant if the 2 values were in the same risk category and discordant when 2 values represented different risk category.

Results: Among the 36 patients, 19 patients had Oncotype Dx® RS tested for one primary cancer, and 17 patients had testing from bilateral primary cancers. Patients median age is 55 years (range: 44 years to 75 years), and invasive duct cell was the most common histology. The RS distribution of low risk, intermediate risk and high risk was 60 %, 30% and 10%, respectively. Further, evaluation of the 17 patients in whom the RS score from bilateral invasive breast cancers was obtained, we noted that the RS was concordant in 11 patients (64.7%), and discordant in 6 patients (35.3%). In 4/6 patients the discordance was between low and intermediate risk, and in 2/6 patients it was between low and high risk. Clinical variables including age, histology, receptor positivity, and grade were not predictive of the rate of RS concordance between the 2 primary cancers.

Conclusion: The significant rate of discordance observed in our study suggests that the 21-gene RS for each invasive primary breast cancer should be routinely obtained in patients presenting with bilateral breast cancers. Albeit synchronous events, the individual risk assessment of each primary cancer would help guide risk-tailored personalized cancer treatment.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-32

Title: The association of breast cancer subtype and breast cancer survival with parity and time since last birth

De Mulder H, Laenen A, Wildiers H, Punie K, Poppe A, Remmerie C, Nevelsteen I, Smeets A, Van Nieuwenhuysen E, Van Limbergen E, Floris G, Vergote I and Neven P. KU Leuven - University of Leuven, University Hospitals Leuven, Leuven, Vlaams-Brabant, Belgium; Interuniversity Centre for Biostatistics and Statistical Bioinformatics, Leuven, Vlaams-Brabant, Belgium; KU Leuven - University of Leuven, University Hospitals Leuven, Leuven, Vlaams-Brabant, Belgium; KU Leuven - University of Leuven, University Hospitals Leuven, Leuven, Vlaams-Brabant, Belgium; KU Leuven - University of Leuven, University Hospitals Leuven, Leuven, Vlaams-Brabant, Belgium; KU Leuven - University of Leuven, University Hospitals Leuven, Leuven, Vlaams-Brabant, Belgium; KU Leuven - University of Leuven, University Hospitals Leuven, Leuven, Vlaams-Brabant, Belgium and KU Leuven - University of Leuven, University Hospitals Leuven, Laboratory of Translational Cell & Tissue Research and University Hospitals Leuven, Leuven, Vlaams-Brabant, Belgium.

Body: Background: Pregnancy affects breast cancer risk but its influence on breast cancer subtype and prognosis remains controversial. We studied the effect of parity and time since last birth on breast cancer subtype and outcome in women aged ≤ 50 years at diagnosis.

Patients and Methods: A retrospective multivariate cohort study including all premenopausal women aged ≤ 50 years (N=1306) at diagnosis and primarily treated with surgery (N=1176) or neo-adjuvant chemotherapy (N=130) at University Hospitals Leuven (Jan. 2000 – Dec. 2009); local and systemic therapies were consistent with guidelines when treated. Tumor subtypes were defined by tumor grade and receptor expression for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) amplification; ER+PR+/-HER-2- cases were Luminal A- like if grade 1-2 and Luminal B like if grade 3; HER-2+ cases were Luminal HER-2 if ER+ and HER-2 like if ER-; triple negative breast cancer (TNBC) were ER-PR-HER-2-. Outcome endpoints were breast cancer subtype, disease free (DFS) and distant disease free survival (DDFS) by parity and in parous women comparing short (< 5 years) versus long (≥ 5 years) time since last birth. Statistics used were Cox proportional hazard model. Results were corrected for age at diagnosis, tumor size, lymph node status and tumor subtype.

Results: Breast cancer subtypes didn't differ between nulliparous and parous women but subtypes differed significantly in parous women by time interval since last birth ($p < 0.001$). Breast cancers within 5 years of last birth were proportionally more likely TNBC and HER-2 like compared to Luminal A ($p = 0.026$ and $p = 0.003$ respectively) than breast cancers ≥ 5 years after last birth even when corrected for age at diagnosis. After a mean follow-up period of 10 years, parous women had a better DFS compared to nulliparous women (DFS: HR 0.754; CI 0.593-0.959; $p = 0.021$) but after correction for known prognostic factors, only a trend remained (HR 0.783; CI 0.611-1.004; $p = 0.054$). In parous women, those with a longer time interval since last birth had a better DFS than women with a recent pregnancy (HR 0.965; CI 0.948-0.982; $p < 0.001$). However, after correction for known prognostic factors, this association was completely attenuated (HR 0.997; CI 0.972-1.023; $p = 0.828$). Comparable results were seen for DDFS.

Conclusion: After correction for age at diagnosis, parity does not but recent birth does affect breast cancer subtype. Such tumors are proportionally more likely ER-negative namely TNBC and HER-2 like. We observed a trend for better DFS for parous women. The prognostic value of time since last birth is mostly due to tumor characteristics and age at time of diagnosis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-33

Title: CASCADE study: Rapid survival decline per treatment line in metastatic breast cancer

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Body: BACKGROUND: Appraisal of the impact that current therapeutic strategies of advanced breast cancer (ABC) have on the survival expectancy, is vital to understand the prognosis of this disease. This entails assessing simultaneously the tumour phenotype and the therapeutic approach used per line of treatment. CASCADE is an epidemiological, retrospective, multicenter study aimed to retrieve demographic and clinical information from a representative cohort of ABC patients treated within the Spanish National Healthcare System.

MATERIAL AND METHODS: 13 Spanish public hospitals serving nearly 5M inhabitants (~10% of the national population) identified 422 ABC patients between 01/2007 and 12/2008 who received active treatment for their disease and were followed until death, lost to follow-up, or until December 2013. Overall Survival (OS) was analysed per tumour immunotype and treatment line from the diagnosis until a minimum of 10 patients were still evaluable. OS resulting from the different therapeutic approaches per line was also revised. Data collected included demographical, pathological, diagnostic, and therapeutic information for the entire follow-up. Descriptive statistics were applied (Methods previously described in SABCS 2015 Poster P3-07-39).

RESULTS: Remarkably, by the 2nd line of treatment, on average, one third of the OS is already gone. Tumour type imposes clear differences in this decline rate. As expected, triple-negative patients have the shortest survival expectancy at diagnosis, but their OS attrition rate is the slowest compared to the other subgroups (Table 1).

Table 1. OS per tumour type and line of treatment in ABC.

Population	OS from ABC Diagnosis (months)	OS from 1L (months)	OS from 2L (months)	OS from 3L (months)	OS from 4L (months)	OS from 5L (months)
Whole (N=422)	33.5	32.6	22.6	16.6	13.5	13.3
HER2-/HR+ (N=187)	38.6	37.1	22.4	15.6	12.6	10.2
Triple-negative (N=67)	19.0	16.5	15.8	14.1	10.2	9.5
HER2+/HR+ (N=72)	34.4	33.7	29.4	21.6	20.3	18.9
HER2+/HR- (N=53)	36.3	35.4	23.1	13.1	9.3	14.1

OS time derived from the five mayor therapeutic approaches used at any given line, could only be registered for chemotherapy, hormone therapy and chemo + anti-HER2 therapy. Regardless of their treatment history, patients treated exclusively with hormone therapy portray a less aggressive behaviour than those treated with chemotherapy only, resembling the natural history of HER2-/HR+ and triple-negative phenotypes (Table 2).

Table 2. OS per pharmacological approach and line of treatment in ABC.

Pharmacological treatment	OS from 1L (months)	OS from 2L (months)	OS from 3L (months)	OS from 4L (months)	OS from 5L (months)	OS from 6L (months)	OS from 7L (months)
Chemotherapy (N=155)	25.0	12.5	13.3	10.8	8.3	10.8	7.2
Hormone therapy (N=92)	44.0	30.9	22.3	11.2	14.0	-	-
Chemo + Anti-HER2 thp. (N=57)	36.9	27.2	18.8	14.1	25.8	-	-
Chemo + Other Targeted thp. (N=44)	19.7	21.0	14.1	21.1	-	-	-
Chemo + Hormone thp. (N=38)	44.3	25.0	-	-	-	-	-

Other Targeted thp.: anti-angiogenic antibody, mTOR inhibitor, anti-EGFR antibody, etc.

CONCLUSION: Chances to benefit from effective treatments may be jeopardized if their start is postponed to late lines. Only the most widely used therapies and, ultimately chemotherapy, hold until very late in the treatment of the advanced disease.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-34

Title: Clinical and pathological characteristics of a representative cohort of long-term metastatic breast cancer (MBC) survivors

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Body: Background: Approximately 5-10% of women with MBC survive 5 years or more. Long-term MBC survival has been attributed to hormone receptor positivity, non-visceral metastases, and oligometastatic disease. The exact characteristics that predict long-term survival have still yet to be clearly elucidated. Here we describe the clinical and pathological characteristics of a representative cohort of long-term MBC survivors.

Methods: All patients diagnosed with MBC who were entered into the MBC database at the Magee Womens Cancer Program of UPMC between January 1999 and March 2016 were evaluated (N=1,232); follow-up information was collected through May 2016 and those who survived ≥ 5 years were included in the analysis.

Results: In total, 238 (19.3%) patients met our inclusion criteria. Median follow-up time was 7.1 years (range: 5.0-17.3), and 81 patients were still alive (34%; median follow-up: 8.5 years) while 157 were deceased (66%; median follow-up: 6.7 years). The majority were Caucasian (N=220; 92.4%) with mean age at diagnosis of MBC of 54.5 years (± 12.6 ; range: 28.2-87.6). Ductal carcinoma was the most common primary cancer (N=177; 74.4%) and 65 (27.3%) women were diagnosed with de novo metastatic disease. Among the primary tumors, 179 (75.2%) were ER- or PR-positive, 76 (31.9%) HER2-positive, and 12 (5.0%) triple negative. Disease burden at metastatic disease presentation was classified according to 2 schemes: a traditional classification of non-visceral involving no vital organ (N=142; 59.7%) vs. visceral (N=96; 40.3%) as well as a more modern classification of limited stage being one site only (N=171; 71.8%) vs. extensive disease being in multiple sites (N=67; 28.2%). Of the patients classified with extensive disease, there were 22 (32.8%) who had disease at 3 or more sites. Lines of systemic chemotherapy received in the metastatic setting were as follows: 0 (N=51; 21.4%), 1 (N=44; 18.5%), 2 (N=35; 14.7%), 3 (N=21; 8.8%), 4 (N=23; 9.7%), and more than 5 (N=64; 26.9%). Additional treatments given included HER2 targeted therapy (N=94; 39.5%) and hormonal therapy (N=205; 86.1%). See Table 1 for additional characteristics by tumor status.

Table 1: Selected characteristics by tumor status

	HR+ (N=179)	HER2+ (N=76)	Triple negative (N=12)
Mean age (\pm SD) at MBC	54.9(± 12.3)	51.4(± 10.9)	49.5(± 16.6)
Median follow-up time, years	7.0	7.1	9.2
Cancer type, N(%) ductal	137(78.7)	66(88.0)	10(83.3)
De novo metastatic disease, N(%)	51(28.5)	27(35.5)	5(41.7)
Disease burden, N (%) visceral	66(36.9)	38(50.0)	4(33.3)
Disease burden, N (%) extensive	44(24.6)	27(35.5)	5 41.7)

Conclusions: In this cohort, the majority of patients had hormone receptor positive tumors and received hormonal therapy. There was a small subset of long-term survivors who had triple negative disease. Disease burden at metastatic presentation was more commonly non-visceral or limited. Interestingly, there was a large percentage of patients who were classified as having visceral or extensive disease at 3 or more sites. Additionally, there were a number of patients receiving multiple lines of therapy suggesting a benefit to therapy. Many of the patients in our cohort had a long-term survival despite having risk factors that have been previously associated with a poor outcome.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-35

Overall p value

Title: Proposal for a new breast cancer staging classification: Incorporating clinical and biologic factors

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Body: Background: The current breast cancer staging system, based on anatomy, does not always reflect the variable clinical course outcomes seen in the clinic. Other important and known determinants of prognosis and survival in breast cancer are age, grade, and receptor subtypes. In this analysis, we sought to demonstrate that these additional factors were important determinants of breast cancer specific and overall survival with an intention to propose a new staging classification.

Methods: Through a prospectively maintained electronic database at the University of Texas MD Anderson Cancer Center, we identified patients with newly diagnosed invasive breast cancer, stage I-IV, who received surgery as an initial treatment from 1997 to 2014. Data points for the earliest invasive breast cancer event were recorded: age, pathologic stage (7th edition AJCC), grade, ER status, PR status, HER2-neu status, adjuvant treatment history, and outcomes (breast cancer-specific survival [BCSS] and overall survival [OS]). Cox proportional hazards model was used for the statistical analysis.

Results: Of 22,131 patients, 99% were women in the following age groups (median age at surgery, 53 years [range, 16-98 years]): age < 40 (13%), 40-69 (76%), ≥70 (11%). Pathologic stages were: I: 50%, II: 39%, III: 9% and IV: 2%; 768 (3.5%) patients had bilateral breast cancer. Biological subtypes were as follows: Triple-negative (TN): 6%, Hormone receptor-positive/HER2-negative (HR+/HER2-): 70%, HER2-positive (HER2+): 24% (HR+, 9%; HR- 15%). Median follow-up was 7.9 years (95% CI, 7.8-8.0). In multivariate Cox regression modeling, age, grade, and clinical biomarker-based subtypes were significantly associated with breast cancer specific survival (BCSS).

Table 1. Breast cancer specific-survival: Multivariate model

Covariate	Level	HR	95% CI (p value)	
Age at Diagnosis	Less than 40	1.52	1.37-1.68 (<.0001)	
	40-69	Reference		<.0001
	70-79	1.05	0.89-1.24 (0.55)	
	Over 80	1.15	0.79-1.66 (0.47)	
Pathologic Stage	IA	Reference		<.0001
	IIB	0.88	0.58-1.32 (0.54)	
	IIA	2.20	1.96-2.46 (<.0001)	
	IIB	3.45	3.06-3.89 (<.0001)	
	IIIA	4.29	3.70-4.96 (<.0001)	
	IIIB	3.43	2.45-4.79 (<.0001)	
	IIIC	6.58	5.52-7.84 (<.0001)	
	IV	15.12	12.72-17.96 (<.0001)	
Biologic Subtype	HR+, HER2-	Reference		<.0001
	HR+, HER2+*	0.58	0.46-0.73 (<.0001)	
	HR-, HER2+*	1.10	0.90-1.35 (0.35)	
	TN**	2.00	1.82-2.21 (<.0001)	
Nuclear Grade	I	Reference		<.0001
	II	1.73	1.34-2.23 (<.0001)	
	III	3.29	2.55-4.24 (<.0001)	

*All patients were treated with trastuzumab in the adjuvant setting **Considering TN as the reference (HR (95% CI): HR+/HER2- (0.50 (0.45-0.55)), HR+/HER2+ (0.29 (0.23-0.37)), HR-/HER2+ (0.55(0.45-0.68)). Abbreviations - BCSS: HR: hazard ratio, CI: confidence interval, HR+: hormone receptor positive, HR-: hormone receptor negative, HER2+: Her2-neu positive, HER2-: HER2-neu negative, TN: triple negative, Reference: 1.00

Conclusion: More individualized prediction of outcomes for breast cancer is possible by considering clinical and biologic characteristics in addition to anatomic stage. We intend to integrate pathologic stage, age, and biologic factors into a novel prognostic model to propose a new staging classification for breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-36

Title: Tamoxifen resistance: EGFR expression in hormone receptor-positive and HER2 negative breast cancer

Bae SY, Nam SJ, Lee SK, Kim SW, Lee JE and Yu JH. Samsung Medical Center.

Body: Purpose: Crosstalk between growth factor receptor tyrosine kinases (RTKs) and the estrogen receptor (ER) represents one of the most important mechanisms of endocrine resistance. EGFR and HER2 have been recognized as prominent factors associated with endocrine resistance. Most previous studies did not identify subgroups by HER2 overexpression and/or included breast cancer with HER2 overexpression. Accordingly, we analyzed HR positive (HR+) tumors without HER2 overexpression (HER2-).

Methods: We analyzed the clinical data of 2,166 patients with HR+HER2- breast tumors, between January 2007 and July 2013. We included only patients who had endocrine therapy with tamoxifen. Immunostaining for EGFR was interpreted as positive when at least 10% of the tumor cells showed moderate to strong membrane staining.

Results: EGFR expression (EGFR+) was present in 109 patients (5%). EGFR expression was significantly associated with more advanced stage and higher grades. In the univariate analyses, EGFR+ tumors were associated with poorer prognosis than EGFR- tumors (5-year DFS, EGFR+ vs. EGFR-, 91.2% vs. 96.6%, $P < 0.001$; 5-year OS, EGFR+ vs EGFR-, 93.1 % vs. 99.4%, $P < 0.001$). In the multivariate analysis, EGFR+ tumors had a hazard ratio of 2.63 (95% CI 1.14 -6.05) for DFS. EGFR+ tumors had a hazard ratio of 8.8 (95% CI 2.68-132.25) for OS.

Conclusion: EGFR expression could be prognostic factor in hormone receptor-positive and HER2 negative breast cancer, for tamoxifen resistance.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-37

Title: Ductal carcinoma in situ: Patient outcomes and association with hormone receptors

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Body: Background

Local recurrence is a major concern in patients (pts) diagnosed with ductal carcinoma in situ (DCIS). Therefore, the need to identify pts at risk for DCIS recurrence is a significant priority. In invasive breast cancers, ER+/PR- subtype is considered a more aggressive tumor phenotype with poorer prognosis as compared to ER+/PR+ tumors. It is unclear whether this molecular subtype holds the same significance in pts with DCIS.

Methods

We designed an analysis to determine if a significant difference exists in the recurrence rates in pts with ER+/PR- DCIS when compared to ER+/PR+ tumors. Six hundred and ninety three pts diagnosed and treated for DCIS at Froedtert & MCW Clinical Cancer Center from Feb 2002-March 2015 were included in our study. Recurrence was defined as either non-invasive or invasive ipsilateral, contralateral or distant disease. Probabilities of recurrences were calculated using Kaplan-Meier estimator. Cox proportional hazards model was used to evaluate the effect of prognostic factors on DCIS recurrence.

Results

Median follow up was 5.2 years. Five year recurrence free survival (RFS) was 91% (95% CI 88.2-93.3) while estimated 7 year RFS was 86% (95% CI 81.9-89.2). Patient characteristics are shown in table below.

Patient characteristics

Characteristics	N (%)
Total no. of patients	693
Median age	53 (21-91)
Median BMI	27 (17-65)
Post-menopausal	480 (69)
OCP use	301 (43)
HRT use	201 (29)
Tumor characteristics	
Median size	0.8cm (0.2-6.5)
Histology	
Solid	349 (52)
Cribriform	290 (43)
Papillary	35 (5)
Comedo necrosis	423 (61)
ER/PR status	
ER+/PR+	482 (71.2)
ER+/PR-	77 (11.4)
ER-/PR-	118 (17.4)
Tumor grade	
Low	125 (18)
Intermediate	305 (45)
High	250 (37)
Treatment	

Lumpectomy	517 (75)
Mastectomy	169 (25)
Radiation	450 (66)
Endocrine therapy	286 (42)
Patients with recurrence	75 (11)
Type of recurrence	
In-situ	44 (6)
Invasive	31 (5)

BMI=body mass index, OCP=oral contraceptive pills, HRT=hormone replacement therapy

Seventy five pts were found to have a recurrence during their follow-up. Most of the grade 1 tumors were ER+/PR+ whereas almost all of the ER-/PR- subtype were high grade tumors. ER+/PR- tumors were mainly intermediate and high grade ($p < 0.0001$). Pts with ER-/PR- tumors had a significantly higher risk of recurrence (HR 3.7, 95% CI 1.9-7.2, $p=0.0001$) and those with ER+/PR- tumor subtype did not have a statistically significant difference in risk of recurrence when compared to ER+/PR+ tumors (HR 1.75, 95% CI 0.92-3.32, $p=0.085$). Pts who did not receive endocrine therapy for their ER+ DCIS had a significantly higher risk of recurrence as compared to those who received endocrine therapy (HR 2.2, 95% CI 1.23-3.92, $p=0.0073$). When compared to pts undergoing lumpectomy and radiation, pts undergoing lumpectomy alone had a significantly higher risk of recurrence (HR 2.5, 95% CI 1.32-4.93, $p=0.005$) whereas those who underwent mastectomy had a significantly lower risk of recurrence (HR 0.34, 95% CI 0.15-0.8, $p=0.014$).

Conclusion

ER+/PR- subtype was not a significant predictor of recurrence in DCIS patients. This finding is in contrast to the risk of recurrence and tumor aggressiveness seen in invasive breast cancers. Mastectomy and post lumpectomy radiation were associated with improved outcomes as was adjuvant endocrine therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-38

Title: Interaction between body mass index and hormone receptor status as a prognostic factor in node-positive breast cancer

Chung IY, Lee JW, Lee JS, Park YR, Lee Y, Lee SB, Kim HJ, Ko BS, Son BH and Ahn SH. Asan Medical Center, Seoul, Republic of Korea.

Body: Purpose: The aim of this study was to investigate the interaction between BMI at breast cancer diagnosis and the various factors including hormone-receptor, menopausal and nodal status, and to find a specific subgroup where BMI has an effect on breast cancer prognosis.

Methods: We retrospectively analyzed the data of 8,763 non-metastatic invasive breast cancer patients from the Asan Medical Center's research database. Overall survival (OS) and breast cancer-specific survival (BCSS) among BMI groups were compared using the Kaplan-Meier method and Cox proportional hazard model with interaction term.

Results: Only in node-positive breast cancer, there was a significant interaction between obesity ($\text{BMI} \geq 30.0 \text{ kg/m}^2$) at diagnosis and positive hormone receptor which showed worse overall survival (OS) and breast cancer specific survival (BCSS) than normal weight patients (adjusted hazard ratio [HR] = 1.65, 95% confidence interval [CI] = 1.01 to 2.69 and HR = 1.90, 95% CI = 1.15 to 3.15, respectively). Underweight ($\text{BMI} < 18.50 \text{ kg/m}^2$) which interacted with negative hormone receptor status in node-positive breast cancer was associated with decreased OS (HR = 2.01, 95% CI = 1.02 to 3.98) and BCSS (HR = 2.15, 95% CI = 1.08 to 4.26). There was no significant interaction between BMI and hormone receptor status in node-negative setting and BMI did not interact with menopausal status in any population.

Conclusions: BMI interacts with hormone receptor status in node positive setting, thereby playing a role in the breast cancer prognosis.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-39

Title: The role of quantitative estrogen receptor status in predicting breast tumor response to neoadjuvant chemotherapy

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Body: Introduction:

Patients with Estrogen Receptor negative breast cancer (BC) are known to have higher tumor response rates than ER positive patients when treated with neoadjuvant chemotherapy (NCT). Few studies have assessed ER status as a quantitative continuous measure in predicting tumor response in this setting.

We aimed to study the association between quantitative ER status and tumor response at surgery in BC patients treated with NCT at our institution, and identify potential predictors of better survival outcomes.

Methods:

A retrospective review using a neoadjuvant BC database (The "Sunnybrook Biomatrix") identified 304 eligible patients that were included in the analyses. A univariate followed by a multivariable logistic regression analyses were conducted to assess the association between quantitative ER (expressed in percentage) and tumor response (good vs. poor response defined as $<$ vs. \geq 50% reduction in tumor size) while controlling for potential confounders.

For the secondary outcome, the Kaplan Meier method was used to estimate the recurrence free survival (RFS) in this cohort. Predictors of RFS were identified using a cox proportional hazards model (CPH) to adjust for clinically relevant variables. A log-rank test was used to compare RFS between groups for any significant binary predictor.

Results:

The median follow up of all patients was 43.3 months (Q1-Q3: 28.7-61.1). Quantitative ER was inversely associated with tumor response in a multivariable logistic regression model (Odds Ratio 0.99 95%CI: 0.99-1.00, $p=0.027$). A cut-off of 60% seemed to best predict the association based on the c-statistic ($c=0.67$) and the receiver operating characteristic curve.

However, quantitative ER was not associated with RFS; pathologic complete response (pCR) was shown to be an independent predictor of RFS in a CPH model (Hazard Ratio: 0.17, 95% CI: 0.07, 0.43, $p=0.0002$) in all patients, after controlling for potential confounders. At 5 years, 93% of patients with pCR and 72% of patients with residual tumor (no pCR) were recurrent-free respectively (log-rank test $p=0.0012$).

Conclusion:

This study suggests that BC patients with ER status $<$ 60% are more likely to respond to NCT. Although ER status itself did not predict for relapse-free survival, patients with a pCR had better RFS, and this association was seen amongst all tumor phenotypes.

The role of quantitative ER in predicting and maximizing tumour response to NCT (including optimizing pCR rate) needs to be better defined in prospective studies.

Key words: Estrogen receptors, breast cancer, quantitative, tumor response, pathologic complete response.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-40

Title: Her2 and hormonal receptor analysis in breast cancer synchronous node metastases could add therapeutic information

Rizzo MM M, Mando P, Perez de la Puente C, Maino M, Ponce C, Fabiano V, Pombo MT T, Loza CM M, Costanzo MV V, Nervo A, Nadal J, Colo F and Chacon RD D. Instituto Alexander Fleming, Ciudad Autonoma de Buenos Aires, Argentina.

Body: Introduction: Breast cancer is the tumor with highest incidence and mortality in women in Argentina. Hormonal receptors (HR) and HER2 are the most important prognostic and predictive factors. It is suggested that estrogen receptors (ER), progesterone receptors (PR) and HER2 expression can vary during tumor progression and metastases development. Published evidence has reported discordance between primary tumors and its metastases ranging from 15-54% for HR and HER2 overexpression. A similar discordance situation may exist among primary tumor (PT) and its axillary nodal metastases (NM)

Objective: To describe the expression and discordance of ER, PR and HER2 in PT and its synchronous NM

Material and methods: Prospective analysis of surgical breast cancer patients (pts) at Instituto Alexander Fleming (Sept 2013 to Feb 2016). HR were analyzed by immunohistochemistry (IHC) according to CAP protocols; values $\geq 10\%$ were considered positive. HER2 overexpression was defined by 3+ IHC or positive FISH. Paired t test was used to compare mean using Prims 5 software.

Results: In 587 breast cancer surgeries, 190 pts presented NM. HR and HER2 analysis was performed in 101 paired samples. Median age was 51 years (r 25-83). Mean tumor size was $3,3 \pm 0,2$ cm. 0,99% (1) were stage I, 68,3% (69) stage II and 30,7% (31) stage III. Most frequent histologic subtype was ductal carcinoma (66.3%). 78.2% presented lymphovascular invasion. Median positive nodes were 2 (r 1-21). Paired samples allowed ER analysis in 99 cases, PR in 98 and HER2 in 96; 8 NM were Her2++; FISH analysis was inconclusive in 4 of them due to insufficient tissue. No differences were observed on ER between PT and NM (72% vs 71.1%, OR: 0,91, IC95 -3.2-5.1; $p>0,05$), nor PR expression (52.1% vs 54,5%, OR: -2.1, IC95 -7.1-3.01; $p>0,05$). PT were ER+ in 86.1% of the cases; NM were ER+ in 82.2%. Regarding PR, PT were 74.3% PR+ and NM were 76.2% PR+. 28 of the 101 pts (27.7%) were discordant. 5 pts with ER+ PT had ER- NM, and 3 pts had PT ER- but ER+ NM, representing a 7.9% of discordance. 4 of 75 pts with PR+ in PT were PR- in NM, while 8 of 26 pts were PR- in PT but PR+ in NM. Of the 13 HER2+ pts, 2 (15%) did not present overexpression in NM. On the other hand, 6 (7%) of the 83 HER2- PT were HER2+ in NM. 4 of 9 pts had PT RH and Her2- (TN), but NM RH and/or Her2+.

Discussion: Tumor heterogeneity is an important issue that may affect clinical decisions. Axillary nodes are the first metastatic site for breast cancer and different studies have shown discrepancy between PT and NM. We described a discordant expression in ER, PR and HER2 of 8%, 12% and 9% respectively. These differences could be due to technical reasons, intratumor heterogeneity and/or a different cellular phenotype. Although the clinical importance of these changes are not completely understood, NM status could bring important prognostic and therapeutic information defining a particular group of patients in which a specific therapeutic strategy could influence long term results. In fact, in our series 44% of TN tumors presented NM with a therapeutic target, either HR or HER2. We therefore confirm that pathological analysis of NM may bring up additional information that could be useful in a prognostic or therapeutic point of view.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-41

Title: Factors predicting treatment outcomes of angiosarcoma of breast: A 25 year single institution experience

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Body: Background: Primary (PAS) and secondary angiosarcoma (SAS) of breast account for < 1% of all breast neoplasms. SAS develops following radiation therapy (RT) to the breast or chest wall for treatment of breast cancer. The rarity of this tumor type makes it challenging to determine prognostic factors and develop optimal treatment strategies. Historically, large NCI cancer centers have reported a median overall survival ranging from 28 – 100 months with recurrence rate of 55%. Methods: We reviewed demographic, tumor, and treatment characteristics of breast AS patients diagnosed and treated at our institution between 1990 and 2015. Overall (OS) and recurrence free (RFS) survival were compared using standard statistical methods at a significance level of 0.05. Results: Of 12155 breast cancers, 22 patients (0.008%) with AS (PAS in 34%, SAS in 66%) were identified. Median age of PAS patients was significantly lower than SAS – 45 vs 71 years ($p < 0.001$). Median tumor size was 6.9 cm (7.3 cm vs 6.9 cm, $p = 0.93$) with multifocal disease seen in 22.7%. Tumor was high grade in 14 (50% vs 83.3%, $p = 0.34$). Median time from RT to SAS diagnosis was 7.8 years. Treatment included: mastectomy in 17 (77.3%), wide excision in 4 (18.2%), adjuvant RT in 4 (18.2%), taxane based chemotherapy in 11 (50%) and chemo-RT in 1 (4.5%). No significant differences were noted in tumor ($p = 0.9$) or treatment characteristics ($p = 0.4$) between PAS and SAS. Recurrence rate (mainly distant) was 36% (8 pts). The 5-year OS and RFS rates were 51% (95% CI 27–72%) and 36% (95% CI 14–58%) with estimated medians of 64.2 and 55.5 months, respectively with no significant difference between the two groups. Black ethnicity (11.6 vs 64.2 months, $p = 0.015$), multifocal disease (15.5 vs 64.2 months, $p = 0.004$) and tumor size of > 6.9 cm (8.3 vs 64.2 months, $p = 0.03$) were associated with poorer outcomes. Tumor grade was not related to OS. Adjuvant treatment (RT $p = 0.49$, chemotherapy $p = 0.36$) conferred no RFS or OS benefit. Conclusions: Patients with PAS were younger than SAS. Black race, multifocal presentation and larger tumor size predict worse clinical outcomes. Our institutional experience confirms the poor prognosis of angiosarcoma, and highlights the need for further research.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-42

Title: The role of serum neutrophil-to-lymphocyte ratio (NLR) in triple-negative breast cancer

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Body: Purpose

Neutrophil-to-lymphocyte ratio (NLR) is known as a predictor of mortality in solid cancers including gastrointestinal cancer, lung cancer, pancreas cancer and hepatocellular carcinoma. In breast cancer, NLR is associated with predicting mortality in patient population. However, there are no reports about clinical usefulness of NLR in triple negative breast cancer (TNBC) patients. In our analysis, we analyzed the clinical association of NLR in TNBC patients.

Methods

Between Jan. 2010 to Dec 2014, 260 patients diagnosed as TNBC by pathologic review were analyzed. Initial complete blood count (CBC) during the first diagnosis of TNBC was collected in total patient population. Among patients receiving neoadjuvant chemotherapy, initial CBC records before administration of systemic chemotherapy and CBC records achieved after the maximal response of systemic chemotherapy were retrieved. In metastatic TNBC patients, initial CBC and follow up CBC during each line of systemic chemotherapy was collected. The clinicopathologic characteristics and survival outcomes [overall survival (OS), relapse free survival (RFS), stage IV OS] were analyzed.

Results

The median age was 51 years (range 14~84). Initial NLR was associated to clinical TNM stage and Ki-67 index, with statistical significance ($P=0.011$ and $P=0.021$, respectively). Patients with lower NLR ($NLR < 2.8$) showed longer OS compared to patients with higher NLR ($NLR > 2.8$) (median OS 34.3 months vs. 32.83 months, $P = 0.02$). In 38 recurrent or metastatic TNBC patients, lower NLR ($NLR < 2.8$) group were associated to longer stage IV OS compared to higher NLR ($NLR > 2.8$) group (median stage IV OS 17.38 months vs. 12.87 months, $P = 0.016$). In 36 patients who received neoadjuvant chemotherapy, lower NLR group showed trends for superior RFS, but without statistical significance. Furthermore, patients with decreased NLR after neoadjuvant chemotherapy also showed trends for superior RFS, but without statistical significance.

Conclusion

In TNBC patients, lower initial NLR was associated with superior survival irrespective of clinical stage. During neoadjuvant chemotherapy, decreased NLR might be associated with superior RFS, but further analysis is warranted. Further studies with sufficient follow-up duration is warranted for validation of the role of serum NLR in TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-43

Title: Bcl2 as a long-term prognostic factor in invasive lobular carcinoma of the breast

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Body: Background: B-cell lymphoma 2 (Bcl2) is an anti-apoptotic protein with known tumor suppressor effect in breast cancer. Multiple studies have shown that high Bcl2-expression is associated with a better prognosis in breast cancer, but its clinical usefulness as a prognostic factor is still not fully elucidated. Invasive lobular carcinoma (ILC) of the breast is the second most common histologic subtype and comprises 5 to 15 % of all breast cancers. ILC has distinct clinico-pathological features and a specific pattern of recurrence, but treatment strategies are often similar as for the whole group of breast cancers. ILC's are typically estrogen receptor positive (ER+), progesterone receptor positive (PgR+), HER2 negative, low proliferative and histological grade 2. According to the St Gallen surrogate definitions of the intrinsic subtypes of breast cancer most ILC's are classified as 'luminal disease' and predominantly as low-risk 'luminal A-like'. Patients with 'luminal disease' in general and 'luminal A-like' in particular falls into prognostic categories where uncertainty about optimal treatment strategy often arises, as clinicians seek to avoid both over- and under-treatment. Additional prognostic factors are needed in order to further improve risk stratification. The use of multigene assays to better predict recurrence is increasing, but the availability is still limited and the cost is high. Measurement of apoptosis-related factors, such as Bcl2 gene expression is included as a prognosticator in a majority of these tests. The aim of this retrospective study was to evaluate the long-term prognostic effect of Bcl2 in a subset of patients with ILC.

Patients and methods: One hundred and ninety-two well-characterized patients with primary ILC were included in the present study. Bcl2 was evaluated together with ER, PgR, HER2, Ki67, histological grade, tumor size, nodal status and age at diagnosis. Ninety-two percent of the patients were ER and/or PgR positive and 41% were node positive. Forty-one percent of the patients received adjuvant endocrine treatment and 3% received adjuvant chemotherapy. The median follow-up of the 52 patients still alive was 26 years. Bcl2 was analyzed immunohistochemically on whole sections of tumor tissue and Bcl2-positivity (Bcl2+) was defined as more than 10% cells with stained cytoplasm. Eighty-six percent of the patients (165/192) were Bcl2+. The primary endpoint was breast cancer mortality (BCM).

Results: Overall, 32% (61/192) of the patients have died from breast cancer. Bcl2 (positive vs negative) was a significant prognostic factor for BCM in univariable Cox regression analysis (HR 0.44, 95% CI: 0.23-0.85). Essentially the same Bcl2 effect was seen after multivariable adjustment for ER, Ki67, histological grade, tumor size, nodal status, age at diagnosis and adjuvant therapy (HR 0.33, 95% CI: 0.15-0.74).

Conclusions: Bcl2 is an independent long-term prognostic factor in this subset of patients with ILC. Bcl2 might add new prognostic information based on apoptotic tumor features that could be useful in the clinical treatment decision-making.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-44

Title: Tumor-infiltrating lymphocytes (TILs) is associated with improved overall survival in triple-negative breast cancer (TNBC) patients treated with neoadjuvant chemotherapy (NAC)

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Body: Background: Recent studies suggest that the percentage of TILs is a predictive factor for response to NAC and a prognostic factor associated with long-term disease control in hormone receptor-negative breast cancer. The TILs working group's current recommendation is to evaluate stromal TILs as the principal parameter in future studies. The term lymphocyte-predominant breast cancer (LPBC) can be used as a descriptive term for tumors that contain more lymphocytes than carcinoma cells. Typically, the threshold of stromal lymphocytes for LPBC is around 50% of the stromal surface area. It is unclear if this cutoff will be used in the future as such an intense TIL infiltration in tumors has been reported to be infrequent (~10%). Studies with TNBC have demonstrated increasingly better overall survival (OS) and disease-free survival (DFS) associated with continuous scores of TIL in patients treated with adjuvant chemotherapy. In patients treated with NAC, TILs predicted pathological complete response (pCR). Our goal was to evaluate the impact of TIL on OS in TNBC patients treated with NAC.

Methods: Data from patients with histologically confirmed TNBC treated with NAC from a single institution (A. C. Camargo Cancer Center - ACCCC), between July 2002 and November 2013, were retrospectively collected using electronic medical records. Patients with metastatic disease or in situ carcinoma at diagnosis were excluded. The density of TILs was evaluated in full-face hematoxylin and eosin-stained (HE) slides. Three blinded pathologists made the assessment of each slide, and a consensus on the TIL percentage was achieved. A cut-off of 10% for TIL percentage was employed for OS and DFS calculations, based on technical statistical maximizing log-rank test. We use this cut-off to test the association with pathological pCR rate as well. For pCR rate, we also used a cut-off of 50% (LPBC). We used Chi-square test to evaluate the association with pCR. A p-value<0,05 was considered statistically significant for all tests.

Results: We identified 78 patients that fulfilled all inclusion and exclusion criteria. The median age was 42 years (range 17-70), and the clinical stage distribution was IIA (14%), IIB (22%), IIIA (19%), IIIB (33%) and IIIC (11%). 58 patients had archival FFPE blocks available and suitable for pathological analysis. Median follow-up was 4,1 years. Overall survival in 5 years in this subgroup was 62% (median not reached). 23 (39.7%) tumors had TIL> 10%, however only 10 had TIL > 50%. TIL >10% was associated with improved OS (HR 0.33, 95% CI, 1.0 to 0.11; p = 0.04). The same cut-off was associated with better DFS, although not statistically significant (HR 0.46, 95% CI, 1.1 to 0.18; p = 0.1). The overall pCR rate was 39.6% (48% for patients with TIL > 10% and 34% for patients with TIL <or = 10%; p = 0.3). LPBC had similar pCR rate (40% for LPBC vs. 39% for non-LPBC), probably due to the small number of samples analyzed. pCR was associated with a decreased risk of death (HR 0.06, 95% CI, 0.008 to 0.47; p<0.01).

Conclusion: We observed improved OS associated with TIL>10% in TNBC patients treated with NAC. pCR was also associated with better OS.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-45

Title: Long-term follow-up of early stage breast cancer patients with results of MammaPrint®, Oncotype DX® and MammoStrat® risk classification assays

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Body: Introduction: The use of genomic tests for the prediction of breast cancer recurrence is becoming more common. MammaPrint® (MP, Agendia Inc.) is a 70-gene microarray assay designed to assess the 10-year risk of recurrence in an untreated population that was not selected for ER/HER2 results. The Oncotype DX® Recurrence Score® (RS, Genomic Health, Inc.) is a 21-gene RT-PCR assay that is clinically validated to predict the 10-year risk of distant recurrence in ER+ patients treated with Tamoxifen. MammoStrat® (MS, Clariant, Inc.) is an IHC assay that uses 5 antibodies and has been validated in a similar population as RS. Several recent reports show that these assays classify patients differently with significant discordances for all risk groups (Shivers, et al., SABCs 2013; Denduluri, et al., ASCO Breast 2011; Poulet, et al., SABCs 2012; Schneider, et al., ASCO 2013). The present study is an analysis of long-term follow-up in a cohort of patients who have results for all three of these risk-stratifying assays side by side in the same samples.

Methods: Patients with ER+ N0-N1 early-stage breast cancer with an MP result obtained as part of their routine clinical care were identified at the University of South Florida (USF, N=65) and Morton Plant Hospital (N=83). After local IRB approval, slides and/or blocks were cut and de-identified at USF and sent to Genomic Health and Clariant for blinded testing. Clinicopathological features were also reviewed by 3 breast pathologists.

Results: 148 patients with an MP result had tissue available to send for RS and MS assays. These patients had a median age of 62 years; median tumor size 1.8 cm; 9% low grade, 59% intermediate grade and 32% high grade. In our previous analysis of this study, of 148 patients with MP results, 53% were low risk and 47% were high risk. Of 135 samples that yielded enough RNA to produce an RS result, 53% were low risk, 26% were intermediate risk and 21% were high risk. Of 129 samples that yielded an MS result, 44% were low risk, 28% were moderate risk and 28% were high risk. Of 121 patients with results for all 3 assays, only 22% were concordant for low risk and 9% were concordant for high risk across all 3 assays. Overall, 30% of cases showed a major discordance such as low risk for one assay and high risk for another. After median follow-up of 54 months, 9 patients have had a distant metastasis and/or 8 patients have died (11 patients total). One patient who had bone metastasis and died had been classified as low risk by all 3 assays. Three patients with distant metastases had a major discordance between assays, with two high risk and one low risk result. Seven patients were classified as high or intermediate/moderate risk by all 3 assays.

Conclusions: This direct comparison demonstrates that although the assays classify a large proportion of patients differently, the patients who ended up with a distant metastasis and/or died of breast cancer had been classified as high risk by at least two of the three assays. This study has important clinical implications since these assays are used to help make treatment decisions regarding which patients might benefit from chemotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-46

Title: A comprehensive analysis of GNAS DNA copy number, levels of mRNA and protein expression in primary breast cancer

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Body: Background: Result of recent advances in genetics, guanine nucleotide binding protein, alpha stimulating (GNAS), transducer of signals from G-protein coupled receptors, has been noted that this factor is related with the onset and progression of tumor in various cancers. We aimed to analyze gene amplification, mRNA and protein expression of GNAS and their potential association with clinicopathological factors and prognosis in primary breast cancer.

Methods: The cohort of this study included 432 primary invasive breast cancer patients treated with standard care at Kumamoto University Hospital between June 2000 and January 2011. We performed a comprehensive analysis of *GNAS* at the levels of gene copy number, mRNA and GNAS protein expression analyzed by qPCR, qRT-PCR and immunohistochemistry (IHC), respectively. In the IHC assessment of GNAS protein expression, an H-score<150 was observed in 191 patients (44.2%), who were defined as having a low protein expression level, and an H-score≥150 was observed in 241 patients (55.8%) who were defined as having a high protein expression level.

Results: The median age at diagnosis was 60 (range 27-93). Three hundred fifteen (72.9%) of these were postmenopausal women. One hundred forty two patients (32.9%) had axillary lymph node metastasis. The median Ki67 labeling index was 23.6 (range 0.5-97.0). The subtypes were 321 ER+/HER2-, 24 ER+/HER2+, 34 ER-/HER2+ and 53 ER-/HER2-. Three hundred twenty one patients (75.9%) were treated with endocrine therapy and 146 patients (34.6%) chemotherapy. Most notably, a low levels of GNAS protein expression was observed in 191(44.2%) patients, and was positivity associated with Ki67 (P=0.028). Furthermore, univariate and multivariate analysis revealed that low GNAS protein expression was significantly related with poor relapse-free survival rate (Log-rank test; P=0.0013, OR:0.40, 05%CI:0.22-0.70) and breast cancer specific survival rate (Log-rank-test; P=0.041, OR:0.43, 95%CI:0.19-0.97). *GNAS* amplification and mRNA expression were not correlated with prognoses.

Conclusion: Contrary to expectations, GNAS expression was positively related with favorable tumor characteristics. Expression levels of GNAS protein may be an independent prognostic factor for primary breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-47

Title: The development of personalized diagnostic tests and therapeutic strategies in breast cancer

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Body: Despite some improvement in the overall survival rates of breast cancer, it remains as a leading cause of cancer-related deaths in women. Currently, we are unable to accurately predict patients' response to therapies and their long-term outcome. We developed and patented a 275-gene signature based on *in silico* meta-analysis of global transcriptome profiles (approximately 10,000 cases) that can predict which patients suffer from aggressive disease and succumb to their disease within 5 years of diagnosis. This test, the integrated Breast Cancer Recurrence (iBCR) score outperformed every clinicopathological indicator available in three independent, large cohorts of breast cancer. The iBCR can also predict the likelihood of response to standard treatments and which emerging targeted therapies should be added to an individual's treatment regime to improve outcomes. In addition, 21 of the genes in this signature are novel potential drug targets that have not previously been described in aggressive breast tumours. We performed a pilot study using the NanoString nCounter Dx platform to measure the expression of the top 125 genes within the signature in a cohort of 48 patients. We have validated with 100% accuracy the prognostic power of the iBCR in the Queensland Follow Up (QFU) cohort with 25 years of follow up ($p < 0.0001$), irrespective of clinicopathological features. Our pilot study using the Nanostring platform successfully counted mRNA molecules from samples that averaged 26.8 years of age (range 24 – 29 years), with an average RNA Integrity (RIN) score of 2.4. Future work will expand this test to the full 275-gene set across 500 patients from the QFU cohort. *In vitro* siRNA screening of the 21 novel genes revealed that at least 10 of these genes are required for breast cancer cell survival. We have started validation of the top 4 hits and these studies confirm the requirement of these genes in breast cancer progression. These data will pave the way towards the study of these genes as new drug targets. Collectively, our test addresses the significant issue of heterogeneous responses to breast cancer treatment. The iBCR platform aims to improve both patients' clinical outcome and quality of life by directing more appropriate treatment with greater likelihood of success, and preventing overtreatment for those with a less aggressive tumor type.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-48

Title: Development of a predictive model by gene expression profiles for pathological CR after neoadjuvant chemotherapy in ER-positive/HER2-negative breast cancer

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Body: Background: Benefits of neoadjuvant chemotherapy (NAC) are tumor shrinkage, improvement of surgical outcome and monitoring of response to systemic therapy. Pathological complete response (pCR) is one of the indicators of the sensitivity to NAC and is associated with improved long-term outcomes. However, response to NAC is different by tumor subtype. It may be difficult to decide the indication to NAC in ER-positive/HER2-negative subtype because pCR rate and its prognostic value is lower than other subtype. In this study, we aimed to develop a predictive model by gene expression profiles for pCR after NAC in ER-positive/HER2-negative subtype.

Patients and Methods: From the previously published 10 microarray data base in Gene Expression Omnibus, we selected the 858 ER-positive/HER2-negative breast cancer patients who had received NAC. Each 10 data sets were analyzed by logistic regression analysis in terms of pCR. Each analyzed data were combined and the 5686 genes were selected as pCR-related genes in ER-positive/HER2-negative breast cancer. Furthermore, we selected top 20 genes related to chemo-sensitivity and top 20 genes related to chemo-resistance. Furthermore, expression levels of each 40 genes were examined by qRT-PCR using archival materials. Finally, predictive model were developed using most predictive genes chosen from these 40 genes. We used 85 formalin-fixed paraffin-embedded tissues from patients with ER-positive/HER2-negative breast cancer before NAC. Chemotherapy included anthracycline and/or taxane. These patients received subsequent surgery at Kumamoto University Hospital between 2004 and 2014. The overall pCR rate was 12.9% (9 patients).

Result: To develop a pCR prediction model, we selected top 8 pCR-related genes based on the expression levels of each genes by qRT-PCR from the 40 genes which were chosen previously published data base. We developed pCR prediction index (pPI) used by univariate and multivariate logistic regression analysis for the expression profile of the 8 genes by qRT-PCR and calculated based on odd's ration with multivariate analysis. pPI was distributed between 7.4 from 1.5E+11. The patients were divided into 2 groups (Low pPI group ; pPI < 32 and High pPI group; pPI \geq 32) according to pPI value. Each pCR rate were 0% (Low; 0/17) and 16.7% (High; 9/54), respectively (p=0.021). pPI was not associated with clinicopathological factors such as age, tumor size, nodal status, nuclear grade, ki67 labeling index. pPI (p=0.0198) was significantly related with pCR by multivariate analysis as well as tumor size (p=0.0136).

Conclusions: We developed pCR prediction model (pPI) based on gene expression levels of 8 genes for ER-positive/HER2-negative breast cancer. pPI may be useful tool for selecting NAC in ER-positive/HER2-negative breast cancer. We need to be validate the pCR predictive value of pPI with another data set.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-49

Title: Three-gene signature predictive of high residual risk of recurrence after adjuvant chemotherapy in ER-positive HER2-negative breast cancer

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Body: Background: A proportion of ER-positive, HER2-negative patients treated with both adjuvant endocrine and chemotherapy continue to remain high risk for recurrence. These individuals are the ideal patient population for future adjuvant trials.

Methods: We used gene expression data from ER+/HER2- patients who received adjuvant endocrine and chemotherapy. We utilized a training cohort of multiple gene expression datasets (N=131; GSE16391, GSE21653, GSE17907, GSE19615, GSE16716, GSE45255) and a validation cohort (N=277; GSE25066) from similarly treated ER+ patients. All data sets were generated with Affymetrix HGU133 arrays. Cox proportional hazards regression was performed across all genes using all possible cutoff values between the lower and upper quartile. Genes were ranked according to the estimated hazard rates for relapse. The top three genes were combined into a final signature that used the mean expression of these genes as the predictive score. Statistical analyses were performed in the R environment.

Results: The final signature capable to predict survival consisted of three genes, MX1, MBD4, and ZWINT. Each of these genes achieved high significance in a univariate analysis: MX1 HR=5.7 ($p=1.8E-04$) and HR=4.7 ($p=1.3E-03$), MBD4 HR=3.4 ($p=2.8E-04$) and HR=4.3 ($p=4.4E-04$), and ZWINT HR=4.4 ($p=1E-03$) and HR=3.7 ($p=8.5E-03$) in the training and validation cohorts, respectively. Using the mean expression of the three genes, the estimated hazard rates for survival were 20.5 ($p=2.5E-05$) and 17.5 ($p=1E-04$) in the two cohorts, respectively. Only one patient relapsed in each cohort. In a multivariate analysis including grade and lymph node status as covariates (size was not available), the three-gene signature retained significance (HR=13.2, $p=0.011$; and HR=13.9, $p=0.01$ in the two cohorts, respectively), while only lymph node status (HR=3.6, $p=0.007$) was significant in the validation cohort and no clinical variable was significant in the training cohort.

Conclusions: High expression of a 3-gene signature can identify ER+ patients who remain at very high risk for recurrence despite current adjuvant endocrine and chemotherapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-50

Title: Impact of young age on recurrence and mortality after surgery in breast cancer: 15 years active surveillance

Kim J-Y, Cho J, Kim H, Kang D, Jung HA, Lee S-H, Bae S, Yu JH, Lee SK, Kim SW, Lee JE, Nam SJ, Ahn JS, Im Y-H, Guallar E and Park YH. Samsung Medical Center, Seoul, Korea; Center for Clinical Epidemiology, Samsung Medical Center, Seoul, Korea; Samsung Medical Center, Seoul, Korea; SAIHST, Sungkyunkwan University, Seoul, Korea and Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.

Body: Introduction: Substantial efforts have been made to find factors associated with breast cancer (BC) recurrence and mortality after BC treatment. So far TNM stage, ER, PR, and HER2 status are considered as the major predictive markers of BC recurrence and used for treatment decision. However, most of these factors were evaluated independent from other important confounders such as age, stage, and various anti-cancer treatments because they were mostly derived from clinical trials. In Korea, up to 50% of BC patients are premenopausal women, it is not clear how age at diagnosis affect the progression and outcomes of the disease considering all known prognostic factors including TNM stage, ER, PR, and HER2 status. We aim to evaluate the impact of young age on recurrence and mortality after surgery among Korean women with BC.

Methods: This is a retrospective cohort study conducted using the data from BC registry from 2000 to 2016 at Samsung Medical Center, Seoul, Korea. Patients who received curative BC surgery and who had histologically-confirmed invasive BC between 2000 to 2011 were included in the study. Patients who second primary cancer or double primary cancer were excluded. Information local, regional, or distant recurrence and death until May 2016 was collected using electronic medical records and National Health Statistics. Cumulative incidence rates of distant recurrence and mortality at 3-years, 5-years and 10-years were calculated using a competing-risk model. Cox proportional hazards analysis were conducted with 3 different models to take into account for potential confounding factors including age, body mass index (BMI), stage and subtype at breast cancer diagnosis, chemotherapy, radiotherapy and hormone therapy.

Results: There were 7360 BC patients with curative BC surgery between 2000 and 2011, and the average follow up duration was 75.4 months. The mean age at diagnosis was 48.4 years old (Standard deviation (SD)=±10), and 6.2% (n=459) was diagnosed younger than 35. Of total, 13.3% were stage III BC and 73.4% of patients had hormone receptor positive BC. The cumulative incidence (95%CI) of recurrence at 3, 5, and 10 years was 4.4% (3.9-4.9), 7.5% (6.8-8.2), and 14.8% (12.9-16.7) respectively. The incidence of mortality at 3, 5, and 10 years was 1.8% (1.5-2.1), 3.8% (3.3-4.3), and 10.2% (9.1-11.5) respectively. Patients who were diagnosed BC under 35 years of age had 2.14 (95% confidence interval (CI):1.74-3.10) and 1.62 (95% CI:1.02-2.56) times higher risk of distant recurrence and mortality compared to patients whose age at diagnosis were between 50 to 60 after adjusting all well-known prognostic factors including stage, subtype, and BMI at diagnosis, chemotherapy, radiotherapy and hormone therapy.

Conclusions: Young age at diagnosis (<35) was the most significant predictor on BC recurrence and mortality independently from BC stage and subtype. Further study is warranted to explain biologic background for the differences in outcomes in young women with BC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-51

Title: The REQUITE-AB study: Validating predictive models and biomarkers of radiotherapy toxicity to reduce side-effects and improve quality of life in breast cancer patients

Rattay T, Johnson K, Azria D, Chang-Claude J, Davidson S, Dunning A, de Ruyscher D, Guterrez-Enriquez S, Lambin P, Rancati T, Rosenstein B, Seibold P, Symonds RP, Thierens H, Valdagni R, Vega A, Webb A, Wenz F, West C and Talbot C. University of Leicester, United Kingdom; University of Montpellier, France; German Cancer Research Centre (DKFZ), Heidelberg, Germany; The Christie Hospital Foundation NHS Trust, Manchester, United Kingdom; University of Cambridge, United Kingdom; University Hospitals Leuven/KU Leuven, Belgium; Vall d'Hebron Institute of Oncology, Barcelona, Spain; MAASTRO Clinic, Maastricht, Netherlands; Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; Icahn School of Medicine at Mount Sinai, New York, NY; Universiteit Ghent, Belgium; Fundacion Publica Galega Medicina Xenomica, Santiago de Compostela, Spain; University Medical Centre Mannheim, Germany and University of Manchester, United Kingdom.

Body: Clinically significant side-effects from radiotherapy affect around a quarter of breast cancer patients and may impact considerably on outcomes from treatment. An increasing number of replicated genetic associations for radiotherapy toxicity are being reported[1]-[2]. The international REQUITE consortium aims to validate genetic markers and clinical factors implicated in radiotoxicity. The purpose of the REQUITE-AB project is to develop an integrated set of predictors for acute radiotherapy side-effects in breast cancer patients to be used as a clinical decision-making tool.

As part of the REQUITE prospective cohort study, 2,000 patients eligible for adjuvant breast radiotherapy will be recruited in nine centres across Europe and North America between April 2014 and August 2016, with centralised data management, biobanking and two years' follow-up using a standardised data collection protocol. Patient characteristics and treatment details being captured also include dose-volume histograms and DICOM files. Genotyping will take place in fall 2016. Primary endpoints are acute skin toxicity (CTC-AE v4.0) and quality-of-life (QoL) on completion of radiotherapy and at 3 months from start of radiotherapy. Secondary endpoints are late side-effects including change in breast appearance.

1,766 breast cancer patients have been recruited to date with standardized documentation of toxicity and QoL. Among patients who completed radiotherapy so far, 21.6% of patients developed grade 2 skin toxicity (brisk erythema) and 1.3% grade 3 (moist desquamation). The ability of patient, treatment and genetic variables to predict clinical outcomes and QoL will be examined. The REQUITE study includes the largest radiogenomics cohort of breast cancer patients to date recruited under a single standardised protocol. Findings of the REQUITE-AB project are likely to inform the development of interventional biomarker trials and personalise breast cancer care in the future.

[1] Talbot et al, *Br J Cancer*, 2012; 107: 748-53.

[2] Seibold et al, *Int J Radiat Oncol Biol Phys*, 2015; 92: 1084-92.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-52

Title: Prognostic factors in breast cancer (BC) associated with the development of brain metastases (BM): A retrospective study of 726 pts

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Body: BACKGROUND: BC is the 2nd commonest cause of BM. These are associated with considerably poorer prognosis. This study aims to define prognostic factors of brain dissemination in patients with limited BC.

METHODS: We retrospectively analyzed 726 pts with non-metastatic BC, treated between 2008 and 2013 in a single-center. Tumour and patient characteristics were reviewed and correlated to the development of BM, other systemic progression, locoregional failure and survival data available. Univariate (UV) and multivariate (MV) Cox regression analyses were performed.

RESULTS: Median follow-up was 40 months. The majority (66%) of tumours were luminal (RE or RP+ / Her2 -), 9% luminal/Her2+ (RE or RP+ / Her2 +), 3% Her2+ (RE and RP- / Her2 +), 12% triple negative (RE and RP- / Her2 -) and 10% not specified. Median patient's age was 60 years.

DFS at 5 and 10 years was 86% (95%- confidence interval [CI]: 82-90%) and 48% (CI: 34-62%), respectively. OS was 92% (CI: 89-95%) and 62% (CI: 50-74 %), respectively. CSS was 94% (CI: 91-97%) and 64% (CI: 51-77%), respectively. LRC was 97% (CI: 95-98%) and 91% (CI: 84-98%), respectively. BM- free survival was 97% (CI: 96-98%) and 73% (CI: 60-86%), respectively. Other distant metastasis control rates were 90% (CI: 87-93%) and 42% (CI: 25-59%), respectively.

Out of 726 patients, 28 developed BM. Their median OS was 41 months. Median time from initial diagnosis to BM was 59 months; and median time from other metastasis to BM was 13 months. In MV, prognostic factors of BM development were primary tumor >T1, lymphovascular invasion (LVI) and Her2+ or triple-negative ($p < 0.05$) breast subtype (BS). These prognostic factors were also significant for DFS, along with >N1. Percentage increase in progesterone and oestrogen receptor positivity was significantly associated with less BM occurrence ($p < 0.0001$). DFS and OS were also significantly influenced by primary tumor >T1, LVI and Her2+ or triple-negative BS and hormonal receptor percentage expression.

CONCLUSIONS: The risk of BM in early-stage BC remains low, but our study found a substantial variation in risk by BS, tumour size and hormonal receptor positivity. These factors might represent prognostic factors to be considered for early detection and/or prevention of BM.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-53

Title: Targeting RB loss for the precision treatment of triple negative breast cancer

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Body: A precision approach to the treatment of TNBC has been woefully lacking, and the vast majority of cases are still treated based on cytotoxic chemotherapy. In the genetic (n=215) and histological (n=220) analysis of TNBC approximately 30% of cases exhibit loss of function of the RB tumor suppressor. This event deregulates cell cycle regulatory processes and is associated with the aberrant expression of a number of genes that are known drug targets. Through the screening of >1,000 compounds with multiple cancer cell lines including naturally occurring and CRISP/CAS9 deletions we observed that RB loss results in a specific profile of sensitivities related to select chemotherapies and drugs targeting DNA-damage checkpoints (e.g. CHK1) and chromosome segregation (e.g. PLK1). These data agree with the known functions of RB in controlling cell cycle regulatory processes and are consistent with analysis of clinical specimens indicating a role for RB in the response to neoadjuvant chemotherapy. Taking advantage of these findings with single agents, we delineated unique combination treatments that were particularly active in RB-deficient TNBC models and could serve as the basis for a precision-guided approach to treatment based on loss of this critical tumor suppressor.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-54

Title: Long-term recurrence risk and risk-based follow-up after breast cancer treatment

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Body: Background and aim: The Dutch guideline prescribes an age-based policy following the first five years of annual follow-up after curative treatment for breast cancer: women aged <60 years should still be seen annually, 60-75 years biannually and >75 years follow-up could be stopped. In this nationwide population based study we analysed long-term breast cancer recurrence patterns and modelled the age-based hazard of recurrence to support an adapted follow-up frequency.

Patients & Methods: Women diagnosed with primary invasive first breast cancer (M0) in 2003/2005 treated with curative intent, were selected from the Netherlands Cancer Registry (N=18,570). Missing data were multiple imputed. Extended Cox regression was performed to identify prognostic factors for locoregional recurrence and determine the hazards for the current age groups. Using the Logrank test, alternative cut-offs for age were determined by testing for equality of the survivor functions (events predicted vs observed) per five-year age intervals. Based on the hazards of the new age groups, thresholds were chosen and applied on two risk profiles as an illustration of the effect of the new thresholds.

Results: During ten years of follow-up, 658 (3.5%) patients developed a locoregional recurrence as a first event. Multivariable analysis showed that young age, greater tumour size, higher grade, positive lymph nodes, multifocality and no treatment with chemotherapy were prognostic factors for recurrence. The hazard after five years of follow-up for women aged <60 years was 0.70%, for 60-75 years 0.76% and for >75 years 0.58%

Age groups (after completing five years of follow-up) and corresponding hazards

Follow-up recommendation	Current age groups	Hazard at year 5 (%)	Alternative age groups	Hazard at year 5 (%)*
Annual	<60	0.70	<50	0.78
Biannual	60-75	0.76	50-80	0.72
Consider stopping	>75	0.58	>80	0.38

* Used as thresholds

. This means that the group of women advised to continue annual follow-up after five years (<60 years) actually have lower risks than women with an advice to visit once every two years (60-75 years).

Testing resulted in alternative cut-offs for age; <50; 50-80, >80 years resulting in distinguishing hazard levels (table). As an illustration, the effect of using these new hazard levels as stopping-thresholds was investigated on two risk profiles based on age and endocrine therapy (ET). Based on the new hazard levels, a redistribution of the visits is suggested over the subsequent five-year follow-up period. The lower risk group (>50 years, with ET) was below the 'consider stopping' threshold and the higher-risk group (<50 years, without ET) was advised to receive six yearly follow-up visits.

Conclusion: The current follow-up policy uses suboptimal age cut-offs, as especially young patients (<50 years after five years of follow-up) have higher risks for locoregional recurrences and 50-60 year old patients have comparable risks as 60-80 year old patients. The proposed alternative cut-offs support a more risk-based follow-up frequency and more efficient allocation of resources. However, to get towards truly personalised follow-up, more variables should be taken into account to provide individualised risk estimates and follow-up schedules, also taking into account the risk on second primary tumours.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-55

Title: "Quality, not quantity": 10X hot-spot (HS) analysis of lymphocyte markers (CD3, CD8, CD4, CD20) in tumor-infiltrating lymphocytes (TILs) is superior to whole tumor (WT) analysis in triple-negative breast cancer (TNBC)

Cui X, McIntire PJ J, Ginter PS S, Irshaid L, Chen Z and Shin SJ J. Weill Cornell Medicine, New York, NY.

Body: Background TILs have emerged as a prognostic indicator of disease-free survival (DFS) and overall survival (OS) in TNBC. Defined as all mononuclear cells within the tumor, TILs are mostly composed of CD3+ cells with the majority co-expressing CD8+ and less CD4+. Many studies have shown a positive effect of CD8+ TILs, however, the data regarding CD4+ TILs are conflicting. Few studies have assessed CD20+ TILs. Surprisingly, the majority of these published studies were performed on tissue microarrays, a method which is least likely to be representative of TILs. In this study, we aimed to evaluate an alternative approach to assessing lymphocyte markers in TILs compared to the gold standard of whole tumor (WT) analysis using imaging analysis software. **Design** Immunohistochemistry (IHC) for CD3, CD4, CD8, and CD20 was performed, each on one representative whole tissue section from 76 cases of primary TNBC. Imaging and quantification of WT and 2.2 mm diameter (equivalent to one 10X field) of highest immunoreactivity (HS) for stromal and intratumoral CD3+, CD4+, CD8+, and CD20+ TILs were performed using HALO™ imaging analysis software (Indica Labs; Corrales, NM). Statistical analyses were performed using DFS and OS (range: 16 to 196 months, mean: 110 months) as primary endpoints and results from the HS versus WT (gold standard). **Results** CD3+ and CD8+ TILs were significantly correlated with DFS using either WT (CD3: $P=.0221$, CD8: $P=.0114$) or a 10X HS field (CD3: $P=.0063$, CD8: $P=.0058$). In addition, CD4+ TILs was significantly correlated with DFS using a 10x HS field ($P=.0231$) For OS, evaluating a 10X HS field for CD3+ and CD8+ TILs was found to be statistically significant (CD3: $P=.0400$, CD8: $P=.0381$) while none was found in any of the markers studied when using WT. CD4+TILs were only observed to be significantly associated with DFS using a 10X HS field ($P=.0231$). CD20 did not correlate with outcome using either method. **Conclusion** In theory, enumeration of immunoreactive cells by image analysis on WT should represent the gold standard in assessing TILs, however, we found that analyzing a single, smaller (10X) field found to have the highest concentration of immunoreactive cells (HS) to be a better predictor of long-term clinical outcome of TNBC. Our findings support the use of HS evaluation in the assessment of immunoreactivity of TILs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-56

Title: Tissue microarray (TMA) based immunohistochemical studies of lymphocyte-specific markers yield inaccurate results

McIntire PJ J, Ginter PS S, Irshaid L, Cui X, Chen Z and Shin SJ J. Weill Cornell Medicine, New York, NY.

Body: Background Tumor-infiltrating lymphocytes (TILs), in particular cytotoxic (CD8+) T-cells, have been associated with increased survival in patients with triple-negative breast cancers (TNBC). A surprising majority of immunohistochemical studies of TILs have been performed using a TMA, typically consisting of 0.6 to 1.5 mm diameter tissue cores. While not explicitly stated, it is unlikely that these TMAs were made for the purposes of studying lymphocyte specific markers and, thus, inaccurately capturing the extent of TILs given their heterogenous distribution near tumor cells. The purpose of this study was to identify the smallest area of analysis necessary to accurately assess a lymphocyte specific immunohistochemical marker while maintaining a prognostic value based on the hypothesis that a TMA core is too small for accurate analysis of this kind. **Design** IHC for CD8 was performed on a TMA slide containing 76 cases of primary TNBC and the corresponding whole tissue slides. Imaging and quantification of combined stromal and intratumoral CD8+ T-cells was performed using HALO™ imaging analysis software (Indica Labs; Corrales, NM) on the following sized areas of analysis: 0.6 mm diameter TMA core, 1.1 mm diameter (equivalent to 20X field), 2.2 mm diameter (equivalent to 10X field), 6 mm diameter field and whole tumor. Evaluation of the 1.1 mm diameter, 2.2 mm diameter, and 6 mm diameter areas was performed in the same region which was visually determined to have the highest proportion of CD8+ TILs (“hot-spot”). Statistical analyses were performed using disease-free survival (DFS) (range: 16 to 196 months, mean: 110 months) as a primary endpoint of 76 TNBC cases. **Results** In all sized areas of analysis performed on the whole tissue section (1.1 mm, 2.2 mm, 6 mm and whole tumor), increased CD8+ TILs were significantly correlated with DFS ($P = .0033, .0058, .0111, \text{ and } .0114$, respectively), and additionally, the 2.2 mm (10X) “hot-spot” field was significantly correlated with OS ($P = 0.0381$). CD8+ TILs analyzed on the TMA core were not found to be significantly correlated with DFS ($P < .05$).

Conclusion Using HALO™ Image analysis software, we have objectively quantified CD8+ TILs, a marker shown to have predictive and prognostic significance in TNBC, in graduated areas of tumor. We conclude that immunohistochemical analysis of lymphocyte specific markers in TMA yields inaccurate (underestimated) results which raises concern of the validity of published results using this platform in this investigative setting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-09-57

Title: Stromal density of tumor-infiltrating lymphocytes (TILs): Challenging the 50% threshold that defines lymphocyte predominant breast cancer (LPBC)

McIntire PJ J, Cui X, Ginter PS S, Irshaid L, Chen Z and Shin SJ J. Weill Cornell Medicine, New York, NY.

Body: Background Assessment of TILs as a clinically relevant immunologic marker in breast cancer is becoming more established, particularly in triple-negative breast cancer (TNBC). Typically, thresholds of ≥ 50 or 60% have been used to define LPBC; however, there is no published evidence to indicate that these cutoffs correlate with long-term clinical outcome in TNBC. We sought to determine a clinically relevant threshold for TILs in a cohort of patients with TNBC with long-term outcome. **Design** Histopathologic assessment was performed on a representative H&E slide of 76 primary invasive TNBC cases. Stromal density of TILs defined as a percentage of intratumoral stroma occupied by mononuclear inflammatory cells over the total intratumoral stromal area was recorded in each case at 5% increments. Statistical analyses using disease-free survival (DFS) and overall survival (OS) (range: 16 to 196 months, mean: 110 months) as primary endpoints were performed to determine clinically relevant thresholds of stromal density to define LPBC from non-LPBC. **Results** Using DFS as the primary endpoint in our cohort of TNBC, the ideal threshold of stromal density of TILs for LPBC based on the lowest P value (0.0006) was $\geq 32.5\%$. In addition, a stromal density threshold of $\geq 50\%$ to define LPBC was significantly associated with DFS ($P=0.0149$). When using OS as the primary endpoint in our cohort of TNBC, the ideal threshold of stromal density of TILs for LPBC based on the lowest P value (0.0308) was $\geq 57.5\%$. However, using a stromal density threshold of $\geq 50\%$ to define LBPC was not found to be significantly associated with OS ($P=0.0599$). Additionally, each 5% increase in stromal density of TILs was significantly associated with improved DFS and OS in TNBC [0.04; 95% confidence interval (CI) 0.01–0.33, $P=0.0025$ and 0.09; 95% CI 0.01–0.69, $P=0.0206$, respectively]. **Conclusion** We found that stromal density of TILs correlates significantly with long-term clinical outcome in a dose-response manner in TNBC. Our findings help validate using a higher threshold of a $\geq 60\%$ for stromal TIL density (instead of 50%) to morphologically classify LPBC in TNBC for survival prognostication. Additionally, our results suggest that using a threshold of $>30\%$ for may be useful in predicting recurrence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-10-01

Title: Efficacy and safety of the therapeutic cancer vaccine tecemotide (L-BLP25) in early breast cancer: Results from a prospective, randomized, neoadjuvant phase-II study (ABCSG-34)

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Body: Background: Immune-based therapeutic strategies represent a promising approach in early and advanced breast cancer treatment. MUC1 glycoprotein is overexpressed and aberrantly glycosylated in over 90% of malignant breast cancer. It is involved in oncogenesis and confers resistance to anti-cancer therapies, thus representing a particularly promising target. Tecemotide is a MUC1-based therapeutic cancer vaccine. The aim of this trial was to investigate the efficacy and safety of preoperative tecemotide in primary breast cancer patients receiving neoadjuvant Standard-of-Care (SoC) treatment.

Patients and Methods: 400 patients with HER2-negative early breast cancer were recruited into this prospective, multicentre randomized 2-arm academic phase-II trial. Patients received preoperative SoC treatment with or without tecemotide therapy. Postmenopausal women with E+++ or E++ and Ki67 <14%, and G1,2,X tumors received 6 months of letrozole as SoC. Postmenopausal patients with triple-negative, E- or E+, or E++ and Ki67 ≥14%, and with G3 tumors, and all premenopausal patients received 4 cycles of epirubicin/cyclophosphamide plus 4 cycles of docetaxel as SoC. Patients were additionally randomized to receive reverse or conventional sequence of epirubicin/cyclophosphamide and docetaxel. Primary endpoint was histopathological response measured by Residual Cancer Burden (RCB0/I vs RCBII/III) at the time of surgery. Secondary endpoints included pCR, efficacy of reverse versus conventional sequence chemotherapy, and safety.

Results: We did not observe a significant difference in RCB0/I rates between patients with (36.4%) and without (31.9%) tecemotide in the overall study population ($p = 0.40$), and in endocrine and chemotherapy treated subgroups (25.0% vs 13.3%, $p = 0.17$; 39.6% vs 37.8%, $p = 0.75$). Similarly, addition of tecemotide did not affect overall pCR rates (22.5% vs 17.4%, $p = 0.23$). RCB0/I rates were comparable regardless of docetaxel being given before or after epirubicin/cyclophosphamide (37.2% vs 40.1%, $p = 0.61$). Tecemotide addition was not associated with a worse toxicity profile (178 AEs, 57 SAEs vs 180 AEs, 48 SAEs based on patient incidence).

Conclusion: Immune-based targeting of MUC1 by tecemotide is safe but does not improve RCB and pCR rates in early SoC-treated breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-10-02

Title: MHC-II positive breast tumors are more immunogenic and may preferentially select for LAG-3-positive tumor immune infiltrates

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Body: Background: Lymphocyte-activation gene 3 (LAG-3) is a T-cell checkpoint regulator and a current target in immunotherapy trials. LAG-3's main ligand is MHC class II (MHC-II), to which it binds with higher affinity than CD4. Binding of LAG3 to MHC-II antigen-presenting cells negatively regulates cellular proliferation, activation, and homeostasis of T cells, similarly to CTLA-4 and PD-1, suggesting that antibodies targeting LAG-3 may demonstrate similar anti-tumor immune effects.

Hypothesis: We recently reported an association of MHC-II on tumor cells and its involvement in mediating sensitivity to PD-1/PD-L1 monoclonal antibodies. MHC-II demonstrates a strong bimodal expression pattern on tumor cells from a variety of tissues, including those of the breast. In breast cancer patients, tumor-specific MHC-II expression on TNBCs is correlated with a 'hot' immune environment. We hypothesized that 1) MHC-II expression may drive potent anti-tumor immune responses and 2) MHC-II-positive tumors that generate immunotolerance may develop a specific immune checkpoint dependency on LAG-3, since LAG-3 is the inhibitory receptor for MHC-II-mediated antigen presentation.

Methods: To determine the functionality of MHC-II in driving anti-tumor immune responses, we constitutively expressed the MHC-II master regulator *CIITA* in MMTV-neu mouse tumor cells and determined their ability to form tumors in immunocompetent syngeneic hosts. To evaluate the association of MHC-II+ tumors with LAG-3 expression, we evaluated LAG-3-positivity by immunohistochemistry (IHC) in lymphocytic infiltrates in a series of 111 post-NAC TNBC specimens from patients with residual disease remaining after presurgical chemotherapy. Tumor-infiltrating lymphocytes (TILs) were scored by H&E, PD-L1 and MHC-II (HLA-DR) were scored in the stroma and tumor compartments using automated quantitative immunofluorescence (AQUA).

Results: Enforced expression of MHC-II via constitutive expression of *CIITA* caused rejection in 60% of mice, while only 11% of mice rejected MMTV-neu tumors expressing the vector control (Fisher's exact $p=0.04$). All rejecting mice were immune to rechallenge with parental (non-*CIITA*-expressing) MMTV-neu cells, suggesting a memory effector response.

Clinically, 11/102 patients (10.8%) had LAG-3+ immune cells in their tumor. LAG-3+ tumors were strongly correlated with MHC-II positivity in tumor cells ($p<0.0001$). Presence of LAG-3+ cells also correlated strongly with overall TILs ($p<0.0001$), and PD-L1 expression on TILs ($p<0.02$). Since the likelihood of identifying LAG3+ lymphocytes is confounded by the inclusion of poorly-infiltrated tumors, we performed a subset analysis on only those tumors with substantial TILs ($>20\%$). When this subset was analyzed, LAG-3 positivity retained its association with tumor MHC-II expression ($p=0.0001$), while the association of LAG-3 with stromal PD-L1 was reduced below the level of significance ($p=0.052$).

Conclusions: MHC-II expression causes increased immune activation in breast cancers, consistent with our previous findings. MHC-II positivity in breast tumors may identify a population with preferential dependence on the LAG-3 checkpoint, which may be important for future immunotherapy trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-10-03

Title: KEYNOTE-012: Long-lasting responses in a phase Ib study of pembrolizumab for metastatic triple-negative breast cancer (mTNBC)

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Body: Background: In the multicenter, nonrandomized phase Ib KEYNOTE-012 trial (NCT01848834), the anti-PD-1 antibody pembrolizumab demonstrated promising antitumor activity (18.5% ORR in patients [pts] with measurable disease at baseline as assessed by RECIST v1.1 and central radiology review; 6-mo PFS rate, 24%; 12-mo OS rate, 43.1%; data cutoff date, March 23, 2015) and a manageable toxicity profile as later-line of therapy for previously treated, PD-L1+ mTNBC. Here we present updated data for the mTNBC cohort of KEYNOTE-012.

Methods: Key enrollment criteria were: age ≥ 18 yr; ER-negative, PR-negative, HER2-negative, recurrent or metastatic breast cancer; measurable disease based on RECIST v1.1; ECOG PS 0-1; any number of prior systemic treatments in the metastatic setting; and PD-L1+ tumors (expression in stroma or $\geq 1\%$ of tumor cells by IHC using the 22C3 antibody). Pts received pembrolizumab 10 mg/kg Q2W for 24 mo or until disease progression or unacceptable toxicity. Clinically stable pts with initial evidence of radiographic progression could remain on pembrolizumab until progression was confirmed. Response was assessed every 8 wk by central radiology review based on RECIST v1.1. After pembrolizumab discontinuation, pts were followed every 3 mo until death or withdrawal of consent. OS was estimated using the Kaplan-Meier method.

Results: Thirty two female pts were enrolled. Median age was 50.5 yr (range, 29-72); 46.9% had received ≥ 3 lines of therapy and 25.0% had received ≥ 5 lines of therapy for metastatic disease. As of the data cutoff date of April 26, 2016, median follow-up duration was 10.7 mo (range, 0.4-32.7). Median OS was 10.2 mo (95% CI, 5.3-17.5) and 12-mo OS rate was 41.1%; 25 (78.1%) pts had died. Median PFS was 1.9 mo (95% CI, 1.3-4.3) and 12-mo PFS rate was 15.0%. Of the 5 responders (including 1 complete response [CR] and 4 partial responses [PR]), 3 have had long-lasting benefit from pembrolizumab. The pt with CR discontinued study medication 11 mo after achieving CR and has remained in CR for approximately 15 mo without receiving any additional anticancer treatment. Two pts with PR discontinued pembrolizumab after completing 2 yr of treatment. The first pt has maintained response for 22.7 mo; the second pt had disease progression after 7.7 mo of response and recently restarted pembrolizumab as allowed by protocol. Median duration of response has not been reached (range, 15-58+ wk). Thirty (93.8%) pts discontinued pembrolizumab (27 [84.4%] with progressive disease and 3 [9.4%] for AEs) before reaching 2 yr of treatment. Six (18.8%) pts experienced grade 3-5 treatment-related AEs; there was 1 treatment-related death (disseminated intravascular coagulation with decreased blood fibrinogen).

Conclusions: Pembrolizumab provides long-lasting responses in heavily pretreated pts with mTNBC. Further development of pembrolizumab for treatment of this poor-prognosis pt population is warranted, and a phase II study evaluating efficacy and safety of single-agent pembrolizumab as later line of treatment for mTNBC is ongoing (KEYNOTE-086, NCT02447003).

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-10-04

Title: Determining the optimal vaccination strategy using a combination of the folate binding protein (FBP) peptide vaccine (E39+GM-CSF) and an attenuated version (E39') to maximize the immunologic response in breast cancer patients

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Body: BACKGROUND FBP is overexpressed in 20-50% of breast(B) cancers(Ca) and roughly 90% of endometrial(E) and ovarian (Ov) Ca. E39 (FBP191-199, EIWTHSYKV)+GM-CSF is an HLA-A2 restricted FBP peptide vaccine, which has been shown to generate significant *in vivo* immunologic response(IR) in a phase I/IIa trial in E Ca and Ov Ca patients (pts). There is a risk of inducing immunologic tolerance after multiple inoculations with a highly immunogenic vaccine. Thus, we are investigating a novel vaccination series using combinations of E39 and E39' (EIWTFSTKV, an attenuated version of E39) in a phase Ib, randomized, single-center trial. We are assessing short and long-term IR. Here, we present the initial IR analysis to the primary vaccination series (PVS) within B Ca pts.

METHODS HLA-A2 positive B or Ov Ca pts were enrolled after completion of standard of care therapy and randomized into three arms: EE (6 inoculations of E39); EE'(3 inoculations of E39, then 3 of E39'); or E'E(3 of E39', then 3 of E39). Theoretically, due to lower FBP expression and less aggressive chemotherapy regimens, B Ca pts are more antigen naïve and have a less suppressed immune system. Thus, only B Ca pts were included in this analysis. The PVS includes 6 inoculations total (R1-R6), one every 3-4 weeks, and containing 250mcg GM-CSF+500mcg peptide in the first 5 pts per arm and 1000mcg of peptide in second 5 pts. To assess the *in vivo* IR, local reaction(LR) was measured 48 hours after each inoculation (R1-R6), and delayed type hypersensitivity(DTH) was measured pre-PVS (R0), 1, and 6-months post-PVS (RC1, RC6). *Ex vivo* IR was measured via dextramer assay for E39-specific CD8+ T-cells at R0, RC1, and RC6. Statistical analyses were completed using appropriate tests.

RESULTS Thirty-five B Ca pts were enrolled, with 27 completing the PVS (EE n=10, EE' n=8, E'E n=9). No clinicopathologic differences between groups or significant toxicities > grade 2 were appreciated. LR increased from R1 to R6 in all groups ($\Delta EE=24.80\text{mm}$, $p=0.14$; $\Delta EE'=38.13\text{mm}$, $p=0.07$; $\Delta E'E=8.05\text{mm}$, $p=0.38$), the greatest increase approaching statistical significance in the EE' arm. The only arm with a statistically significant increase for *in vivo* DTH from R0-RC1-RC6 was in the EE' arm ($\Delta EE=-6.17\text{mm}$, $p=0.27$; $\Delta EE'=44.58\text{mm}$, $p<0.05$; $\Delta E'E=-1.42$, $p=0.37$). *Ex vivo* analysis of IR revealed no significant difference between groups at R0($p=0.45$) or RC6($p=0.72$), nor within groups over time (EE $p=0.32$, EE' $p=0.47$, E'E $p=0.30$).

CONCLUSION In this phase Ib trial analyzing the IR of B Ca pts receiving a different vaccination strategy, both peptides were noted to be safe and immunogenic. While no difference was seen in E39-specific CD8+ T cells between groups, the *in vivo* response was enhanced with the use of E39' after E39; this may indicate expansion of more effective clonal populations of CD8+ T cells with this strategy. These results may be specific to B Ca pts who are relatively antigen-naïve with relatively intact immune systems. Further analysis of these pts as this trial continues will determine the optimal vaccination strategy capable of stimulating and maintaining an IR to prevent B Ca recurrence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-10-05

Title: A pilot study of radiation (RT) and CTLA4-mediated checkpoint blockade with tremelimumab for the treatment of breast cancer brain metastases (BCBM)

McArthur HL L, Beal K, Halpenny D, Henrich M, Patil S, Young R, Kaley T, Hamilton N, Hudis C, Wolchok J and Norton L.
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Body: Background: BCBM is a common and morbid complication of breast cancer. Conventional systemic therapies have demonstrated limited effectiveness in preventing and treating BCBM. Whole brain RT (WBRT) and stereotactic radiosurgery (SRS) remain the cornerstone of BCBM management but offer modest benefits. Preclinical and clinical data indicate that the local effects of RT may be augmented by immunotherapy. Furthermore, abscopal, or distant benefits outside of the RT field have been reported when combined with CTLA4-blockade. Thus, a pilot study of standard-of-care brain RT with tremelimumab, a CTLA4-directed monoclonal antibody, was undertaken to evaluate the impact on distant (non-CNS) disease control in women with HER2-normal (HER2-) disease and to confirm safety when administered with HER2-directed therapy (H) in women with HER2-positive (HER2+) disease.

Methods: Eligible women were age ≥ 18 y, ECOG 0-2, with radiologically confirmed BCBM of any histology for which WBRT or SRS was planned, and non-CNS disease progression (PD) or stable disease (SD) for which a change in systemic therapy was planned. Women with HER2- disease were enrolled in an efficacy cohort with a primary endpoint of 12 week non-CNS disease control by RECIST. Women with HER2+ disease were enrolled in a safety cohort and continued H. Tremelimumab (10mg/kg) was administered within 5 days prior or 3 days after the first fraction of RT, then monthly for 6 months, and then every 3 months. Planned exploratory studies included comparison of non-CNS responses by RECIST to immune related Response Criteria (irRC). The study is closed to accrual and intention-to-treat analyses are reported.

Results: Of the 6 women with HER2+ disease: 6 (100%) had WBRT; 1 had a non-CNS partial response (PR) at 12 weeks (56% by RECIST and 86% by irRC) that was durable at 6 months (after PD on 5th line chemotherapy with H prior to enrollment); 1 had PD at 12 weeks; 1 died at 5 weeks; and 3 have not yet reached 12 weeks. Of the 20 women enrolled in the efficacy cohort: 17 (85%) had WBRT; 12 (60%) had estrogen receptor positive (ER+) disease; 1 with ER+ disease had non-CNS SD at 12 weeks and remains on study; 5 had non-CNS PD at 12 weeks; 3 had PD-related death within 12 weeks of enrollment (median survival 6.4 weeks, range 4.7 - 9.6 weeks); and 7 have not yet reached 12 weeks. Among all 26 study subjects, 20 grade 2 and 3 grade 3 potentially attributable toxicity events were reported.

Conclusions: BCBM RT with tremelimumab was associated with acceptable tolerance and few attributable toxicity events. This is a poor prognosis population with 3/13 (23%) PD-related deaths reported within 12 weeks of enrollment in the evaluable HER2- cohort. Only modest activity (SD in 1/13, 8%) has been observed in the HER2- cohort to date. However, 1 woman with HER2+ disease had a non-CNS PR by RECIST at 12 weeks that was durable at 6 months. This is the first report of a durable response with HER2-directed treatment and checkpoint blockade in breast cancer. The contribution of RT to this patient's response is not discernible but because of the potential importance of this potential interaction, studies of immune therapy with HER2-directed therapy +/- RT are planned.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-10-06

Title: Inducing immune responses to tumor associated carbohydrate antigens by a carbohydrate mimetic peptide vaccine: Clinical experience in phase I and phase II trials

Kieber-Emmons T, Hutchins LF F, Emanuel PD D, Pennisi A, Siegel E, Jousheghany F, Karbassi BM M and Makhoul I. Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, AR.

Body: Active immunization of cancer patients to induce de novo functional anti-tumor immune responses is an alternative/complementary approach to chemotherapy. Tumor vaccines hold the potential to deliver durable, specific and systemic anti-tumor responses in patients. We have been developing active vaccination strategies targeting tumor associated carbohydrate antigens (TACAs) using carbohydrate mimetic peptides. TACAs play roles in initiation and metastasis of cancer and considered as common targets shared by many tumor types. TACA support cell survival that can be interrupted by anti-carbohydrate antibodies. An early-phase 3+3 clinical trial was conducted to evaluate the feasibility, safety and immune functionality of a carbohydrate mimetic-peptide (CMP) vaccine referred to as P10s, which can induce TACA reactive, proapoptotic antibodies. In this trial a dose-escalation trial of vaccine plus adjuvant was conducted in two cohorts of 3 subjects each. Patients were restricted to females of all races with histologically or cytologically confirmed stage IV breast cancer who had stable disease and a positive recall-antigen response. P10s was synthesized with the Pan-T-cell epitope PADRE and formulated at 300 and 500 µg/injection with *MONTANIDE*TM ISA 51 VG for the 1st and 2nd cohorts, respectively. Doses of the appropriate formulation of the vaccine were administered to research participants subcutaneously on weeks 1, 2, 3, 7 and 19. Blood samples were collected at various time points and tested for presence and functionality of antibodies. Antibody response to P10s and in particular against the ganglioside GD2 was measured by ELISA. Binding of pre-immune and post-immune sera was assessed against breast cancer cell lines. Vaccination generates IgG response with serum antibodies capable of inhibiting tumor growth in spheroid culture of breast cancer cell lines. The vaccine induced antibodies in all 6 subjects, displaying significant cytotoxic activity against several representative human breast-cancer cell lines. Caspase 3 was involved in the postimmune serum-mediated apoptosis. No cytotoxicity toward a normal breast epithelial cell line was detected. Apoptosis and caspase 3 activation seems to be involved in anti-tumor cell activity. Immunization with the P10s vaccine was found to be safe and tolerable, and induces functional antibodies that potentially have a cell-death-mediated therapeutic benefit. Incubation of spheroids with post-immune serum further sensitized cells to drugs, improving the efficacy of drug treatment at lower doses. The data suggest that the vaccine-induced anti-tumor immune response in combination with standard of care chemotherapy may further improve clinical outcome. Consequently, we are testing the vaccine in a Phase II study in the neoadjuvant setting. 5 Cohorts of 5 patients each administered with the vaccine at different schedules of chemotherapy are being assessed for immune response to the vaccine as in the Phase I study and if the combination approach contributes to a difference in pathological complete response (PCR) from chemotherapy alone.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-10-07

Title: Long survival of patients with locally advanced breast cancer on combined neoadjuvant chemotherapy and immune potentiation: Modern lessons from an old study

Pohlmann PR R, van Crujisen H, Stam AGM GM, Lougheed SM M, van den Eertwegh AJM JM, Hoekman K, Scheper R, Buter J, van der Hoeven JJM JM, van der Wall E, Pinedo HM M and de Gruijl TD D. MedStar Georgetown University Hospital/Lombardi Comprehensive Cancer Center, Washington, DC; Vrije Universiteit Medical Center, Amsterdam, Netherlands; Medical Center Alkmaar, Alkmaar, Netherlands and University Medical Center Utrecht, Utrecht, Netherlands.

Body: Background: Effective neoadjuvant chemotherapy in combination with hematopoietic growth factors may act as an *in vivo* immunization regimen, leading to effective anti-tumor immunity. In addition to a direct anti-tumor effect, the balance between immunostimulatory Dendritic Cells (DC) subsets on the one hand and regulatory T cells (Tregs) and Myeloid Derived Suppressor cells (MDSC) on the other, may be decisive in clinical outcome.

Methods: We studied the effects of neoadjuvant treatment with doxorubicin and cyclophosphamide on these immune effector subsets in peripheral blood of a subgroup of patients (n=16) with Locally Advanced Breast Cancer (LABC) who were enrolled in the Spinoza trial at the VU University Medical Center in Amsterdam, The Netherlands, from Feb/1999 to Dec/2002. Patients were randomized for systemic administration of either GM-CSF (n=10) or G-CSF (n=6). Small sample size precluded multivariate analysis. Age-matched healthy donors were selected from the VUmc tissue bank. All study procedures were approved by the VUmc IRB.

Results: Average tumor size diameter of patients at VUmc site was 8.0cm (median 6.8cm). Pre-treatment frequencies of conventional CD1c/BDCA1⁺ Dendritic Cell (cDC-1) and CD16⁺M-DC8⁺ non-classical monocytes subsets were significantly lower than in 13 age-matched healthy women. Frequencies of the cDC-1 and -3 subsets, as well as of monocytes, MDSC and Tregs increased over treatment with either GM-CSF or G-CSF administration, whereas plasmacytoid DC (pDC) and BDCA3⁺ cDC-2 rates only increased in G-CSF-administered patients. High pre-treatment frequencies of cDC-1, and (non-)classical monocytes, and low (below median) pre-treatment frequencies of Tregs were associated with prolonged overall survival, although patient numbers and events were too small for these associations to reach significance. Despite the observed similarity in systemic effects on immune effector subsets, prolonged disease free ($P=.05$) and overall survival ($P=.15$) was observed for the GM-CSF-administered patients as compared to patients receiving G-CSF. Additional analyses revealed increased numbers of mature migratory cDC in the tumor-draining lymph nodes of GM-CSF- rather than of G-CSF-administered patients ($P=.03$).

Conclusions: Enhanced systemic DC differentiation occurred with effective neoadjuvant chemotherapy supported either by GM- or G-CSF. GM-CSF has additional maturational effects on regional cDC. In this subgroup analysis, treatment with GM-CSF instead of G-CSF in combination with neoadjuvant chemotherapy containing doxorubicin and cyclophosphamide was associated with prolonged disease free survival. Our data supports additional studies with GM-CSF as potential immunotherapeutic strategy for patients with breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-10-08

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-01

Title: Abstract Withdrawn

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-02

Title: Efficacy of Hsp90 inhibitor ganetespib plus standard neoadjuvant therapy in high-risk breast cancer: Results from the I-SPY 2 trial

Forero A, Yee D, Buxton MB B, Symmans WF Fraser, Chien AJ Jo, Boughey JC C, Elias AD D, DeMichele A, Moulder S, Minton S, Kaplan HG G, Albain KS S, Wallace AM M, Haley BB B, Isaacs C, Korde LA A, Nanda R, Lang JE E, Kemmer KA A, Hylton NM M, Paoloni M, van't Veer L, Lyandres J, Perlmutter J, Hogarth M, Yau C, Sanil A, Berry DA A and Esserman LJ J. University of Alabama at Birmingham, Birmingham, AL; University of Minnesota, Minneapolis, MN; University of California, San Francisco, San Francisco, CA; MD Anderson Cancer Center, Houston, TX; Mayo Clinic, Rochester, MN; University of Denver, Denver, CO; University of Pennsylvania, Philadelphia, PA; MD Anderson Cancer Center, Houston, TX; Moffitt Cancer Center, Tampa, FL; Swedish Medical Center, Seattle, WA; Loyola University, Chicago, IL; University of California, San Diego, San Diego, CA; UT Southwestern Medical Center, Dallas, TX; Georgetown Lombardi Comprehensive Cancer Center, Washington, DC; University of Washington, Seattle, WA; University of Chicago, Chicago, IL; University of Arizona, AZ; Oregon Health and Science University, Portland, OR; QuantumLeap Healthcare Collaborative, San Francisco, CA; Gemini Group, Ann Arbor, MI; University of California, Davis, Davis, CA and Berry Consultants, Austin, TX.

Body: Background: Pathologic complete response (pCR) after neoadjuvant therapy is an established prognostic biomarker for high-risk breast cancer (BC). Improving pCR rates may identify new therapies that improve survival. I-SPY 2 uses response-adaptive randomization within biomarker subtypes to evaluate novel agents when added to standard neoadjuvant therapy for women with high-risk stage II/III breast cancer; the goal is to identify regimens that have $\geq 85\%$ Bayesian predictive probability of success (statistical significance) in a 300-patient phase 3 neoadjuvant trial defined by hormone-receptor (HR), HER2 status and MammaPrint (MP). We report the results for Ganetespib, a selective inhibitor of Hsp90 that induces the degradation/deactivation of key drivers of tumor initiation, progression, angiogenesis, and metastasis. Ganetespib + taxanes previously have resulted in a superior therapeutic response compared to monotherapy in multiple solid tumor models including BC.

Methods: Women with tumors ≥ 2.5 cm were eligible for screening and participation. MP low/HR+ tumors were ineligible for randomization. QTcF > 470 msec and HbA1C $> 8.0\%$ were ineligible. MRI scans (baseline, +3 cycles, following weekly paclitaxel, T, and pre-surgery) were used in a longitudinal statistical model to improve the efficiency of adaptive randomization. Ganetespib was given with weekly T at 150 mg/m² IV weekly (3 weeks on, 1 off). Patients were premedicated (dexamethasone 10mg and diphenhydramine HCl 25-50 mg, or therapeutic equivalents). Analysis was intention to treat with patients who switched to non-protocol therapy counted as non-pCRs. The Ganetespib regimen was open only to HER2- patients, and eligible for graduation in 3 of 10 pre-defined signatures: HER2-, HR+/HER2- and HR-/HER2-.

Results: Ganetespib did not meet the criteria for graduation in the 3 signatures tested. When the maximum sample size was reached, accrual stopped. Ganetespib was assigned to 93 patients; there were 140 controls. We report probabilities of superiority for Ganetespib over control and Bayesian predictive probabilities of success in a neoadjuvant phase 3 trial equally randomized between Ganetespib and control, for the 3 biomarker signatures, using the final pCR data from all patients. Safety data will be presented.

Signature	Estimated pCR Rate (95% probability interval)		Probability Ganetespib Is Superior to Control	Predictive Probability of Ganetespib Success in a Phase 3 Trial
	Ganetespib N =	Control N =		
	93	140		
All HER2-	26% (16%-37%)	18% (8%-28%)	91%	47%
HR+/HER2-	15% (4%-27%)	14% (4%-24%)	60%	19%

HR-/HER2-	38% (23%-53%)	22% (9%-35%)	96%	72%
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Conclusion: The I-SPY 2 adaptive randomization model efficiently evaluates investigational agents in the setting of neoadjuvant BC. The value of I-SPY 2 is that it provides insight as to the regimen's likelihood of success in a phase 3 neoadjuvant study. Although no signature reached the efficacy threshold of 85% likelihood of success in phase 3, we observed the most impact in HR-/HER2- patients, with a 16% improvement in pCR rate. While our data do not support the continued development of Ganetespib alone for neoadjuvant BC, combinations with Ganetespib, which could potentiate its effect, may be worth pursuing in I-SPY 2 or similar trials.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-03

Title: A phase 2 open-label study of lucitanib in patients (pts) with FGF aberrant metastatic breast cancer (MBC)

Mayer IA A, Arteaga CL L, Nanda R, Miller KD D, Jhaveri K, Brufsky AM M, Rugo H, Yardley DA A, Vahdat LT T, Sadeghi S, Audeh MW William, Rolfe L, Litten J, Knox A, Raponi M, Tankersley C, Isaacson J, Wride K, Morganstern DE E, Vogel C, Connolly RM M, Gradishar WJ J, Patel R, Puzstai L and Abu-Khalaf M. Vanderbilt-Ingram Cancer Center, Nashville, TN; University of Chicago Medical Center, Chicago, IL; Indiana University Simon Cancer Center, Indianapolis, IN; Memorial Sloan Kettering Cancer Center, New York, NY; University of Pittsburgh Cancer Institute, Pittsburgh, PA; University of California, San Francisco, San Francisco, CA; Sarah Cannon Research Institute, Nashville and Tennessee Oncology, PLLC, Nashville, TN; Weill Cornell Medicine, Iris Center Breast Center, New York, NY; University of California, Los Angeles, Los Angeles, CA; Cedars Sinai Medical Center, Los Angeles, CA; Clovis Oncology, San Francisco, San Francisco, CA; Clovis Oncology, Boulder, Boulder, CO; Dana Farber Cancer Institute, Boston, MA; University of Miami, Deerfield Beach, FL; John Hopkins Kimmel Cancer Center, Baltimore, MD; Northwestern University, Chicago, IL; Comprehensive Blood and Cancer Center, Bakersfield, CA and Yale University, New Haven, CT.

Body: **BACKGROUND:** Lucitanib is a potent, oral antiangiogenic tyrosine kinase inhibitor of Vascular Endothelial Growth Factor Receptors 1-3 (VEGFR1-3), Platelet-Derived Growth Factor Receptors alpha and beta (PDGFR α/β), and Fibroblast Growth Factor Receptors 1-3 (FGFR1-3). FGF aberrancies (amplification of *FGFR1*, or 11q[amplicon containing FGF ligands 3, 4, and 19]), are genomic alterations observed in over 20% of breast cancer pts and promote cancer proliferation and survival.

METHODS: MBC pts who had received at least 1 metastatic line of therapy were randomized 1:1 to 10 or 15 mg QD of lucitanib. Stratification was based on local assessment of FGF aberrancy; pts with both *FGFR1* and 11q-amplified tumors were stratified as *FGFR1* amplified. Central confirmation of *FGFR1* or 11q amplification was done using Abbott FISH probes (*FGFR1* or 11q copy number ≥ 6 and a ratio of *FGFR1* or 11q to centromere ≥ 2). Investigator-assessed progression-free survival (PFS) was the primary endpoint. Secondary endpoints included objective response rate (ORR) per RECIST 1.1, disease control rate (DCR), duration of response (DR), and incidence of treatment-emergent adverse events (TEAE).

RESULTS: Enrollment completed in 3/2016; 178 pts that received at least 1 dose of lucitanib are included in this analysis (baseline characteristics in Table 1).

Baseline Characteristics

	10 mg QD	15 mg QD
	N=109	N=69
Age (years)		
Median	56	53
Range	27-82	27-80
Sex		
Female	109 (100%)	67 (97%)
Male	0	2 (3%)
ECOG PS		
missing	5 (5%)	2 (3%)
0	51 (47%)	30 (43%)
1	53 (49%)	37 (54%)
Number of prior anticancer therapies in the metastatic setting		
> 3	32 (29%)	21 (30%)
3-6	48 (44%)	32 (46%)
> 6	29 (27%)	16 (23%)

Endocrine/HER2 status		
missing	7 (6%)	1 (1%)
ER+ or PR+	74 (68%)	50 (73%)
HER2+	12 (11%)	7 (10%)
TNBC	16 (15%)	11 (16%)
FGFR aberrancy		
FGFR1 amplified	54 (49%)	29 (42%)
11q amplified	31 (28%)	24 (35%)
FGFR1 and 11q amplified	13 (12%)	9 (13%)
FGFR1 and 11q non-amplified	11 (10%)	7 (10%)

Due to grade 3 hypertension in the 15 mg group (46% vs 37% in 10 mg group), enrollment to the 15 mg group was halted. Overall, most pts (97%) experienced at least 1 TEAE, with the most frequently ($\geq 30\%$) occurring events being hypertension (73%), fatigue (48%), nausea (43%), hypothyroidism (40%), and headache (33%). Grade ≥ 3 TEAEs occurred in 66% of pts, with hypertension as the most frequent event (40%) followed by proteinuria and hyponatremia (both 6%). AEs were manageable with dose interruption or reduction, with approximately 8% of pts ending treatment due to an AE. Current median PFS is 3.5 mos (95% CI 2.8-4.6; range 0.62-12.95) and 2.6 mos (95% CI 1.8-2.9; range 0.82-18.87) respectively for the 10 mg and 15 mg treatment groups. No differences in clinical activity were observed by treatment group, FGF aberrancy, hormone receptor or HER2 status. Of the 168 evaluable pts, confirmed ORR was 3%; overall DCR was 27% (32% for pts in the 10 mg group compared to 20% for the 15 mg group); overall mean (standard deviation) DR of 3.3 (1.8) mos.

CONCLUSION: At 10 mg QD, lucitanib has modest activity with manageable toxicity in this heavily pretreated pt population. Future clinical development for lucitanib may focus on alternative biomarkers to identify sensitive tumors and rational combinations with other anti-cancer drugs.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-04

Title: The evaluation of ganitumab/metformin plus standard neoadjuvant therapy in high-risk breast cancer: Results from the I-SPY 2 trial

Yee D, Paoloni M, van't Veer L, Sanil A, Yau C, Forero A, Chien AJ Jo, Wallace AM M, Moulder S, Albain KS S, Kaplan HG G, Elias AD D, Haley BB B, Boughey JC C, Kemmer KA A, Korde LA A, Isaacs C, Minton S, Nanda R, DeMichele A, Lang JE E, Buxton MB B, Hylton NM M, Symmans WF Fraser, Lyandres J, Hogarth M, Perlmutter J, Esserman LJ J and Berry DA A. University of Minnesota, Minneapolis, MN; QuantumLeap Healthcare Collaborative, San Francisco, CA; University of California, San Francisco, San Francisco, CA; Berry Consultants, Austin, TX; University of Alabama at Birmingham, Birmingham, AL; University of California, San Diego, San Diego, CA; MD Anderson Cancer Center, Houston, TX; Loyola University, Chicago, IL; Swedish Medical Center, Seattle, WA; University of Denver, Denver, CO; UT Southwestern Medical Center, Dallas, TX; Mayo Clinic, Rochester, MN; Oregon Health & Sciences University, Portland, OR; University of Washington, Seattle, WA; Georgetown Lombardi Comprehensive Cancer Center, Washington, DC; Moffitt Cancer Center, Tampa, FL; University of Chicago, Chicago, IL; University of Pennsylvania, Philadelphia, PA; University of Arizona, AZ; University of California, Davis, Davis, CA and Gemini Group, Ann Arbor, MI.

Body: Background: I-SPY 2 is a multicenter, phase 2 trial using response-adaptive randomization within biomarker subtypes to evaluate novel agents when added to standard neoadjuvant therapy for women with high-risk stage II/III breast cancer - investigational agent(I) +paclitaxel(T) qwk, doxorubicin & cyclophosphamide(AC) q2-3 wk x 4 vs. T/AC (control arm). The primary endpoint is pathologic complete response (pCR) at surgery. The goal is to identify/graduate regimens that have $\geq 85\%$ Bayesian predictive probability of success (statistical significance) in a 300-patient phase 3 neoadjuvant trial defined by hormone-receptor (HR) & HER2 status & MammaPrint (MP). Regimens may also leave the trial for futility ($< 10\%$ probability of success) or following accrual of maximum sample size ($10\% \leq$ probability of success $< 85\%$). We report the results for experimental arm Ganitumab, a type I insulin-like growth factor receptor (IGF1R) inhibitor. IGF1R inhibitors are known to induce insulin resistance and all patients assigned to Ganitumab received metformin.

Methods: Women with tumors ≥ 2.5 cm were eligible for screening. MP low/HR+ and HER2+ tumors were ineligible for randomization. Hemoglobin A1C $\geq 8.0\%$ were ineligible. MRI scans (baseline, 3 cycles after start of therapy, at completion of weekly T and prior to surgery) were used in a longitudinal statistical model to improve the efficiency of adaptive randomization. Ganitumab was given at 12mg/kg q2 weeks and metformin at 850mg PO BID, while receiving ganitumab. Analysis was intention to treat with patients who switched to non-protocol therapy counted as non-pCRs. Ganitumab/metformin was open only to HER2-patients, and eligible for graduation in 3 of 10 pre-defined signatures: HER2-, HR+HER2- and HR-HER2-.

Results: Ganitumab/metformin did not meet the criteria for graduation in the 3 signatures tested. When the maximum sample size was reached, accrual to this arm stopped. Ganitumab/metformin was assigned to 106 patients; there were 128 controls. We report probabilities of superiority for Ganitumab/metformin over control and Bayesian predictive probabilities of success in a neoadjuvant phase 3 trial equally randomized between Ganitumab/metformin and control, for each of the 3 biomarker signatures, using the final pathological response data from all patients. Safety data will be presented.

Signature	Estimated pCR Rate (95% probability interval)		Probability Ganitumab/ Metformin Is Superior to Control	Predictive Probability of Success in Phase 3
	Ganitumab/ Metformin N = 106	Control N = 128		
All HER2-	22% (13%-31%)	16% (10%-23%)	89%	33%
HR+/HER2-	14% (4%-24%)	12% (4%-19%)	66%	21%
HR-/HER2-	32% (17%-46%)	21% (11%-32%)	91%	51%

Conclusion: The I-SPY 2 adaptive randomization study estimates the probability that investigational regimens will be successful in a phase 3 neoadjuvant trial. The value of I-SPY 2 is to give insight about the performance of an investigational agent's likelihood of achieving pCR. For Ganitumab/metformin, no subtype came close to the efficacy threshold of 85% likelihood of success in phase 3, and this regimen does not appear to impact upfront reduction of tumor burden. Our data do not support its continued development for the neoadjuvant treatment of breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-05

Title: Phase 1 study of CB-839, a small molecule inhibitor of glutaminase (GLS), in combination with paclitaxel (Pac) in patients (its) with triple negative breast cancer (TNBC)

DeMichele AM M, Harding JJ J, Telli ML L, Münster P, McKay RR R, Iliopoulos O, Whiting S, Orford KW W, Bennett MK K, Mier JW W, Owonikoko TK K, Patel MR R, Kalinsky K, Carvajal RD D, Infante JR R and Merit-Bernstam F. University of Pennsylvania, Philadelphia, PA; Memorial Sloan Kettering Cancer Center, New York, NY; Stanford University, Stanford, CA; University of California, San Francisco, San Francisco, CA; Harvard University, Cambridge, MA; Emory University, Atlanta, GA; Florida Cancer Specialists, Sarasota, FL; Columbia University, New York, NY; Tennessee Oncology, Nashville, TN; University of Texas, Houston, TX and Calithera Biosciences, San Francisco, CA.

Body: Background: CB-839 is a first-in-class highly selective inhibitor of GLS, a key enzyme in the utilization of glutamine by cancer cells. TNBC has high GLS expression and is very dependent upon GLS-mediated conversion of glutamine to glutamate for tumor cell growth. CB-839 has antitumor activity in vitro and in vivo in preclinical models of TNBC. Recent studies demonstrate that glutamine utilization can contribute to resistance to paclitaxel, a therapy frequently used to treat TNBC patients. Paclitaxel sensitivity is dependent on down-regulation of the glutamine transporter, SLC1A5, and over-expression of SLC1A5 causes paclitaxel resistance. Consistent with these observations, inhibition of glutamine metabolism with CB-839 has demonstrated strong antitumor activity in combination with paclitaxel.

CX-839-001 is an ongoing Phase 1 trial of CB-839 as monotherapy and in combination with approved agents. We previously reported pharmacodynamic studies demonstrating robust inhibition of GLS in pt blood and tumors and excellent tolerability of CB-839 monotherapy in a variety of tumor types including TNBC. In light of the preclinical rationale and monotherapy tolerability a combination arm was opened testing CB-839 with paclitaxel (Pac-CB) in patients with advanced TNBC. We report here updated results on the Pac-CB dose escalation and expansion cohorts.

Methods: Patients with refractory advanced/metastatic TNBC (prior taxane therapy allowed) received escalating doses of CB-839 (400-800 mg BID) in combination with a fixed weekly Pac dose of 80 mg/m² Days 1, 8, 15 of a 28 day cycle. Upon demonstration of safety and tolerability, an expansion cohort of TNBC pts was opened.

Results: To date, 15 pts have received Pac-CB at three dose levels of CB-839: 7 pts at 400 mg BID, 5 at 600 mg BID and 3 at 800 mg BID with the latter dose level not completed. 40% of enrolled patients have received >5 prior lines of systemic therapy for adv/met disease, and 10 pts have received prior taxane therapy including 5 in the adv/met setting. The Pac-CB combination has been well tolerated with one DLT during dose escalation (G4 neutropenia at 400 mg BID) and a low rate of dose reductions (2 for Pac and 1 for CB-839). Of 15 pts, the best overall response rate (BORR, see Table) has been PR in 20% (3 pts), SD in 47% (7 pts) and PD in 33% (5 pts) with 5 patients remaining on study. At doses ≥600 mg BID (n=8) the BORR is 38% (3 pts), and disease control rate (CR + PR + SD) is 88% (7 pts). All 3 pts with PRs have received prior Pac, including 2 pts with disease that was refractory to Pac in the advanced/metastatic setting.

Conclusions: The Pac-CB combination has been well tolerated and has demonstrated clinical activity in heavily pre-treated pts with TNBC. At doses ≥600 mg BID, BORR has been 38% and DCR 88%. Notably, PRs have occurred in pts with prior Pac therapy, including 2 pts with Pac-refractory disease in the adv/met setting. Updated data on the escalation and expansion cohorts will be presented.

Dose Level	Total	400 mg BID	600 mg BID	800 mg BID
RECIST Response Evaluable (N)	15	7	5	3
PR	3 (20%)	0	2 (40%)	1 (33%)
SD	7 (47%)	3 (43%)	2 (40%)	2 (67%)
DCR (CR+PR+SD)	10 (67%)	3 (43%)	4 (80%)	3 (100%)
PD	5 (33%)	4 (57%)	1 (20%)	0

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-06

Title: Bromodomain inhibitors represent a rational therapeutic option for the treatment of invasive lobular carcinoma

O'Connor DP P, Walsh L, Tarrant F, Chin S-F, Schouten P, Linn S, Bernards R, Caldas C, Gallagher WM M and ni Chonghaile T. Royal College of Surgeons in Ireland, Dublin, Ireland; University College Dublin, Dublin, Ireland; Cambridge University, Cambridge, United Kingdom and Netherlands Cancer Institute, Amsterdam, Netherlands.

Body: Invasive lobular carcinoma (ILC) is the second most common type of breast cancer after invasive ductal carcinoma (IDC), accounting for approximately 10-15% of all breast tumors. ILC is characterized by inactivation of E-Cadherin and neoplastic cells that invade the stroma in a "single-file" pattern. Women with ILC are usually older, have used hormone replacement therapy and are more likely to have hormone receptor-positive disease. ILCs have similar survival to IDCs at both five and 10 years, but despite this, the clinical course is distinct: ILCs are three times more likely to metastasize to the peritoneum, gastrointestinal tract, and ovaries and are more frequently bilateral. Therefore, tailored therapeutic options for this distinct, hard-to-treat subtype of breast cancer are required.

As part of the RATHER FP7 HEALTH consortium (www.ratherproject.com), we carried out RNA-Seq analysis of 61 primary ILC samples and identified that high expression of the BET family protein Brd3 was associated with poor recurrence free survival ($p=0.03$, HR 8.63, CI 1.22-60.85). This observation was further validated in the independent METABRIC cohort ($n=99$), where again, high Brd3 expression was associated with poor recurrence-free survival ($p<0.01$, HR=3.16, CI 1.24-8.03). Using a two ILC cell lines (SUM44PE and MDA-MB134VI) we found that ILC cells were relatively resistant to the anti-estrogen therapies tamoxifen and fulvestrant compared to those derived from IDC. Next, we tested whether the ILC cell lines were sensitive to BET protein inhibition using the pan-BET family inhibitor JQ1. Interestingly, while JQ1 inhibited cell growth in both ILC cell lines tested, apoptosis was only induced in SUM44PE cells, while MDA-MB134VI cells exhibited G1 arrest. Dynamic BH3 profiling was used to dissect the underlying anti-apoptotic dependencies in each ILC cell type and showed that in the JQ1-resistant MDA-MB134VI cells, survival was predominantly Bcl2-dependent. Combination of JQ1 and the Bcl2-inhibitor venetoclax (ABT-199) synergistically killed MDA-MB134V1 cells compared to treatment with JQ1 alone, while combination with the Bcl2/Bcl-XI/Bcl-W inhibitor navitoclax (ABT-263) added further synergy .

With a number of BET inhibitors now entering clinical trials, the data described here suggest that BET inhibition is a rational therapeutic option for some ILC cases, and for those that do not respond, combination with venetoclax may be a suitable therapeutic strategy. In our cell line models, baseline Bcl-2 expression was sufficient to predict induction of apoptosis in response to JQ1 and could be used to guide therapeutic choice. These results should now be investigated *in vivo* before a prospective clinical trial.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-07

Title: Methylation of BRCA1 and response to the PARP inhibitor veliparib

Puhalla SL L, Katz TA A, Diergaarde B, Yu J and Oesterreich S. University of Pittsburgh, Pittsburgh, PA.

Body: Background: The BRCA1 promoter has been demonstrated to be methylated in up to 14% of sporadic breast and up to 30% of sporadic ovarian cancers, resulting in decreased mRNA expression. Drugs targeting tumors that are deficient in DNA repair, such as the PARP inhibitor veliparib, show great promise in BRCA-deficient cancers.

Hypothesis: BRCA1 promoter methylation is an alternative mechanism to mutation resulting in “BRCAness”-associated vulnerabilities that can be clinically targeted.

Methods: BRCA1 methylation was analyzed in samples (n=58 pre-tx samples) from two multi-institutional clinical trials in patients with advanced primary breast and ovarian tumors evaluating veliparib as a single agent (NCT00892736) or in combination with chemotherapy (NCT01281150). Eligibility included advanced malignancy with BRCA germline mutation, or sporadic, BRCA wild type (BRCA-wt) triple negative breast cancer or ovarian cancer. Of the 58 tumors, 35 were BRCA mutation positive (+), and 23 were BRCA-wt/unknown (21 breast/2 ovary). DNA was isolated from FFPE sections using the Allprep kit (Qiagen), bisulfite converted and used for pyrosequencing, covering region -56 to +7 bp covering the TSS of the BRCA1 promoter, and containing 5 CpG sites that were previously shown to be associated with BRCA1 mRNA expression. MCF-7 and HCC38 served as negative and positive controls, respectively. BRCA1 methylation was calculated by averaging the % methylation of the 5 CpG sites, with CpG average % methylation >34.68 (75%ile) considered methylated.

Results: Methylation of the 5 CpG sites tested was concordant amongst sites. The methylation ranged from 6.3% to 47.7%. The mean in the BRCA+ group was 27.96 and 32.29 in the BRCA-wt group, with a trend to more unmethylated tumors in the BRCA+ group. In the BRCA-wt group treated with single agent veliparib or in combination with carboplatinum/paclitaxel, those with higher BRCA methylation were numerically more likely to have response. There was a larger % of BRCA+ or BRCA methylated subjects who had objective response (complete response+ partial response + stable disease) than those who were BRCA-wt and not methylated (59.5% vs 46.2%). This was not statistically significant though likely due to small sample size. In addition, there were 2 patients with pre and post treatment biopsy samples with decrease in methylation after treatment, both of whom had a partial response. The remaining 6 patients with pre- and post-treatment samples had no change in methylation after treatment with veliparib + chemo with some responders and non-responders in this group. One BRCA-wt patient with high BRCA methylation in the tumor had exceptional response > 5 years to chemo + veliparib, followed by maintenance veliparib.

Conclusion: There was evidence of detectable BRCA methylation in patients who were BRCA-wt and some of these patients had response to veliparib. There were clinical objective responses seen in both BRCA methylated and non-methylated patients suggesting that there are additional explanations for BRCAness in this population. Assessment of BRCA methylation status deserves additional evaluation in larger data sets.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-08

Title: Safety and efficacy results from phase I study of BYL 719 plus nab-paclitaxel in HER 2 negative metastatic breast cancer

Sharma P, Abramson VG G, O'Dea A, Lewis S, Scott JN N, Ward J, De Jong JA A, Lehn C, Brown AR R, Williamson SK K, Perez RP P, Komiya T, Godwin AK K, Reed GA A and Khan QJ J. University of Kansas Medical Center, Westwood, KS and Vanderbilt University, Nashville, TN.

Body: Introduction

Mutations/deregulations in the phosphatidylinositol-3-kinase (PI3K) pathway are common in breast cancer, Inhibition of the PI3K pathway is recognized as a promising target for the treatment of breast cancer. Although taxanes are effective early on in advanced stage breast cancer, resistance often develops. It has been demonstrated that activation of the PI3K/AKT pathway confers resistance to paclitaxel, and in preclinical models, concomitant inhibition of the PI3K pathway enhances the efficacy of taxanes. BYL719 is a potent oral, class I PI3K inhibitor which strongly inhibits the PI3K alpha isoforms and is significantly less active against the other class I isoforms. Targeting the alpha isoform of PI3K is expected to improve the therapeutic window over inhibitors with less isoform specificity. Nab-Paclitaxel is a solvent-free, nanoparticle, albumin-based paclitaxel which takes advantage of the antitumor activity of paclitaxel while decreasing the toxicities typically associated with the solvent (Cremophor) used to administer the most common formulation of paclitaxel.

Methods

A 3+3 dose-escalation design evaluated three dose levels of BYL719 (250mg, 300mg, and 350mg) administered PO once daily (D1-28) with nab-Paclitaxel (100 mg/m² intravenously D 1, 8, 15) every 28 days in patients with metastatic HER 2 negative breast cancer. The aims of the study were to 1) determine the recommended phase II dose (RPTD) of BYL719 + nab-Paclitaxel, 2) assess pharmacokinetics of BYL and nab-paclitaxel, and 3) assess preliminary efficacy.

Results

10 patients were enrolled at 3 dose levels of BYL719 and 3 patients were enrolled in expansion cohort at the RPTD of BYL719 of 350 mg PO daily plus nab-paclitaxel 100mg/m² (D 1, 8, 15). Median age was 61years; 54% (7/13) of patients were hormone receptor positive and 46% (6/13) triple negative. 85% (11/13) had visceral disease, 69% (9/13) had received prior chemotherapy for metastatic disease and 85% (11/13) had received prior taxane in adjuvant/metastatic setting. There were no DLTs in the three cohorts and the MTD of BYL was not reached. Hyperglycemia (G3:31%, G4:0%) and neutropenia (G3:15%, G4:8%), were the most common grade 3/4 adverse events. There were no Grade 3/4 diarrhea or rash. Best overall response for 12 patients was 58% (7/12) (complete response=1, partial response=6), and an additional 33% (4/12) demonstrated stable disease. Objective responses were noted in both hormone positive and triple negative disease. Median duration of response is 6.5 months (range 2-14 months). No pharmacokinetic interactions were detected when BYL and nab-paclitaxel were co-administered.

Discussion:

This phase I study demonstrates that combination of BYL719 and nab-paclitaxel was well tolerated and shows encouraging efficacy in metastatic HER2 negative breast cancer. Enrollment in the phase II portion of the trial at the RPTD (BYL719 350mg PO daily plus nab-paclitaxel 100mg/m² D1,8,15 every 28 days) continues. Ongoing analysis of PI3K pathway alterations in tumor and cfDNA will be correlated with clinical response.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-09

Title: Heavily pre-treated breast cancer patients show promising responses in the first in human study of the first-in-class fatty acid synthase (FASN) inhibitor, TVB-2640 in combination with paclitaxel

Brenner AJ J, Falchook G, Patel M, Infante JR R, Arkenau H-T, Dean EM M, Borazanci E, Lopez JS S, Moore K, Schmid P, Frankel AE E, Jones S, McCulloch W, Kemble G, Grimmer K and Burris H. Cancer Therapy and Research Center, San Antonio, TX; Sarah Cannon Research Institute at HealthONE, Denver, CO; Sarah Cannon Research institute, Florida Cancer Specialists, Sarasota, FL; Sarah Cannon Research Institute, Tennessee Oncology, Nashville, TN; Sarah Cannon Research Institute UK, London, England, United Kingdom; The Christie NHS Foundation Trust, Manchester, England, United Kingdom; HonorHealth Research Institute, Phoenix, AZ; The Royal Marsden, Institute of Cancer Research, Sutton, Surrey, United Kingdom; University of Oklahoma Health Sciences Center, Oklahoma City, OK; Barts Cancer Institute, London, England, United Kingdom; Univ. of Texas Southwestern Medical Center, Dallas, TX and 3-V Biosciences, Inc., Menlo Park, CA.

Body: Introduction

FASN inhibition is a novel approach to cancer treatment involving selective disruption of palmitate biosynthesis that, in tumor cells, leads to apoptosis. TVB-2640 is an oral, first-in-class, small molecule reversible inhibitor of FASN that demonstrates *in vivo* antitumor effects. We previously reported the results of dose escalation and now present evidence of preliminary activity in breast cancer patients treated in the dose expansion cohort.

Methods

This ongoing international, multicenter Phase I trial enrolls patients (pts) with advanced solid tumors with adequate organ function. TVB-2640 is given orally once daily at the MTD (100 mg/m²) as monotherapy (mono) or in combination (combo) with weekly IV paclitaxel (80 mg/m²).

Results

The most common AE's observed [mono, N=53; combo, N=47] were: alopecia (57%), palmar-plantar erythrodysesthesia (PPE) (36%), dry eye (13%) and increased lacrimation (11%). Gr. 3 toxicities include corneal edema (3%) and PPE (10%). Other toxicities were ≤ Gr. 2 and only minor GI symptoms occurred. All toxicities were reversible on dose interruption and no enhancement of common paclitaxel toxicity was observed when given with TVB-2640. Rare cases of pneumonitis in combination have been observed but the contribution of TVB-2640 to this effect is uncertain.

14 breast cancer patients were enrolled and treated in combination with weekly paclitaxel while 3 breast patients were given monotherapy (during the dose escalation phase). Among patients treated in combination, three (3) confirmed RECIST partial responses (cPR) were seen and multiple cases of prolonged stable disease (SD) (≥16 wks) despite heavy pre-treatment and taxane resistance in all but 2 cases. One ER+, PR+, Her2+ patient achieved a cPR and was on study for 26 weeks. One ER-/PR-/Her2+ patient whose previous best response to paclitaxel treatment was SD for 24 weeks, reached a cPR at week 12, discontinued paclitaxel at week 21 and remains on monotherapy TVB-2640 with a sustained cPR at week 29. The third responder with cPR is an ER+, PR+, HER2- patient whose previous taxane treatment lasted 15 weeks (response unknown) and she remains on study at week 24. Of the remaining 11 patients, 10 achieved SD > 12 weeks and 8 of the 10 maintained that response for 16-45 weeks. The ongoing SD patient at week 45, discontinued paclitaxel at week 35 and remains on monotherapy TVB-2640.

Summary

TVB-2640 demonstrated multiple cPRs and prolonged SD when combined with weekly paclitaxel in 93% of patients treated. Further exploration of response in patients recently progressed on a taxane (progression within the prior 6 months) and safety with TVB-2640 in combination with docetaxel is being explored.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-10

Title: Preclinical efficacy of the novel PIM2 kinase inhibitor, JP11646 in triple negative breast cancer models

Mehta R, Kothai Guruswamy Sangameswaran D, Bezbatchenko K, Moore J, Gil M, Khoury T, Baldino C, Caserta J, Fetterly, Jr. G, Lee K, Adjei A and Opyrchal M. Roswell Park Cancer Institute, Buffalo, NY; Univ of Texas Medical School at Houston, Houston, TX; Physician Assistant Practice Program, College of Health Sciences and Professions, Ohio University, Dublin, OH; Roswell Park Cancer Institute, Buffalo, NY; Jasco Pharmaceuticals, Woburn, MA; Roswell Park Cancer Institute, Buffalo, NY; Roswell Park Cancer Institute, Buffalo, NY and Mayo Clinic, Rochester, MN.

Body: Background: Triple negative breast cancer (TNBC) patients have poorer prognosis and there remains a lack of novel targeted therapies for their treatment. PIM2 (Proviral Integrations of Moloney virus 2) belongs to a family of three kinases that have been implicated in the survival and progression of hematologic malignancies and solid tumors. PIM2 has been linked to epithelial to mesenchymal transition in TNBC, which can lead to metastasis and chemotherapeutic resistance. We hypothesized that PIM2 may present as a therapeutic target in TNBC.

Materials and Methods: The study involved both *in vitro* and *in vivo* studies involving a novel PIM2 inhibitor JP11646 (obtained from Jasco Pharmaceuticals). TNBC cell lines MDA-MB-231 and BT-549 were obtained for our *in vitro* studies. Cell viability was evaluated using MTT assay. Western Blot assay was used to evaluate relative protein expression. For *in vivo* studies, female SCID mice were inoculated in the mammary fat pads with 1×10^6 MDA-MB-231 cells. When tumor volumes reached 100 mm^3 , the mice were treated with JP11646 at the dosage 15mg/kg intraperitoneally for 2 consecutive days weekly for total of 4 weeks as determined from previous experiments. Control animals received vehicle only. The mice were euthanized once tumors reached $\sim 1,700 \text{ mm}^3$.

Results: BT-549 cells treated *in vitro* with 3 different available PIM kinase inhibitors AZD 1208, LGB321 and JP12641 showed only modest reduction in cell viability. However, treatment of both MDA-MB-231 and BT-549 with JP 11646 demonstrated significant reduction in cell viability with IC50 ranging from 40 to 71.6 nM. Treatment with JP11646 demonstrated a novel mechanism of action resulting in downregulation of PIM2 in both cell lines. Treatment with JP11646, but not other PIM kinase inhibitors, resulted in activation of apoptosis as measured by cleaved PARP (cPARP) levels. Anti-PIM2 siRNA treatment but not treatment with non-specific PIM kinase inhibitor AZD1208 resulted in cPARP induction. Inhibition of proteolysis by bortezomib resulted in preservation of PIM2 and inhibition of apoptosis as demonstrated by decreased cPARP levels after treatment with JP11646. PIM2 over-expressing clone of MDA-MB-231 cells showed enhanced proliferation and migration properties both *in vitro* and *in vivo*. Treatment of mice with orthotopically implanted MDA-MB-231 tumors with JP 11646 resulted in significant reduction in the tumor growth ($p=0.0019$) and increased overall survival ($p=0.018$) as compared to control mice.

Conclusions: PIM2 upregulation in TNBC cell line resulted in more aggressive phenotype. JP11646, through novel mechanism of action resulting in degradation of PIM2, showed robust activity in TNBC cell lines both *in vitro* and *in vivo*. Further correlative studies in tumors harvested from *in vivo* experiments are ongoing. These results encourage further exploration of use of JP11646 as a targeted agent in treatment of TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-11

Title: Abemaciclib exposure-response relationship in patients with metastatic breast cancer in MONARCH 1

O'Shaughnessy J, Chigutsa E, Kambhampati SRP, Sykes A, Frenzel M, Nanda S, Koustenis A, Smith I and Turner PK Kellie. Baylor-Sammons Cancer Center, Dallas, TX and Eli Lilly and Company, Indianapolis, IN.

Body: Objective: Abemaciclib is an oral, selective inhibitor of CDK4 & 6 that exhibited single agent activity in the MONARCH 1 clinical trial¹. The relationship between abemaciclib exposure as monotherapy and response for efficacy and adverse events was evaluated in patients with metastatic breast cancer.

Methods: Abemaciclib (200 mg) was administered orally on a continuous schedule every 12 hours in 28 day cycles until disease progression. Sparse pharmacokinetic samples (PK) were collected, cycle (C) 1 day (D) 1 pre dose, C1D15 4 and 7 hours post dose, C2D1 pre and 3 hours post dose, and pre dose on C3D1. Exposure metrics were predicted from a population PK model and included the minimum concentration after a single dose on D1(C_{min,d1}), maximum concentration at steady state (C_{max,ss}), and minimum concentration at steady state (C_{min,ss}). Exposure to abemaciclib, active metabolites LSN2839567 (M2) and LSN3106726 (M20), and total active species (abemaciclib + M2 + M20) were tested.

Response endpoints including efficacy and adverse events were evaluated 12 months after the last patient entered treatment. The relationship between exposure and objective response rate (ORR) was evaluated by quartile analysis and logistic regression. The relationship between exposure and progression free survival (PFS) was evaluated by Kaplan Meier analysis of exposure quartiles and a Cox Proportional Hazard model with PFS as the response and exposure as a covariate. The relationship between exposure and neutropenia, diarrhea, fatigue, nausea, vomiting, and any treatment emergent adverse event (TEAE) was evaluated by logistic regression on the incidence of any grade, grade ≥ 2 , and grade ≥ 3 events. No adjustments for multiplicity were performed.

Results: Analyses included 132 patients. A summary of the exposures in MONARCH 1 are presented in Table 1. The confirmed ORR was 19.7%, and median PFS was 5.95 months (95%CI: 4.2, 7.5). There was no statistically significant relationship between C_{min,d1}, C_{min,ss}, C_{max,ss} for abemaciclib, M2, M20, or total analyte and ORR or PFS. There were no statistically significant and clinically relevant relationships between abemaciclib exposure and TEAEs.

Conclusion: The results of the exposure-response analysis support the abemaciclib single agent starting dose of 200 mg twice daily and dose reductions in 50 mg decrements as needed for adverse events. Further studies to refine the PK model and evaluate relationships between exposure and response are warranted.

Table 1 Abemaciclib exposures in MONARCH 1

Analyte	Geometric Mean (μM) (CV%)		
	C _{max,ss} (μM)	C _{min,d1} (μM)	C _{min,ss} (μM)
Abemaciclib	0.509 (42.6)	0.136 (54.2)	0.356 (59.9)
M2	0.247 (62.4)	0.0511 (51.4)	0.187 (83.8)
M20	0.395 (61)	0.0687 (56.7)	0.312 (79.9)
Total Active Species	1.19 (43.2)	0.262 (47.6)	0.888 (60.7)

References: 1. Dickler, M. et al. American Society of Clinical Oncology (ASCO), abstract #510 (2016).

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-12

Title: Subgroup Analysis by prior treatment and disease burden in MONARCH 1: A phase 2 study of monotherapy abemaciclib, a CDK4 & 6 inhibitor, in patients with HR+/HER2 metastatic breast cancer (MBC) following chemotherapy

Rugo H, Nanda S and Koustenis A. University of California San Francisco Comprehensive Cancer Center, San Francisco, CA and Eli Lilly and Company, Indianapolis, IN.

Body: Background: The MONARCH 1 (NCT0210249) study demonstrated durable single agent activity of abemaciclib in patients (pts) with HR+/HER2- breast cancer, with an objective response rate of 19.7% and median duration of response of 8.6 months¹. The MONARCH 1 study population was heavily pretreated and had a high disease burden: all pts had previously received endocrine therapy and chemotherapy, had median of 3 prior systemic therapies for metastatic disease 90.2% had visceral disease and 85.6% had ≥ 2 metastatic sites.

Methods: MONARCH 1 was a phase 2 single-arm study designed to evaluate safety and efficacy of abemaciclib monotherapy in women with HR+/HER2- MBC whose disease progressed on/after endocrine therapy and chemotherapy. Abemaciclib (200 mg) was administered orally on a continuous schedule every 12 hours until disease progression. The primary objective planned to evaluate investigator-assessed objective response rate (ORR= complete response [CR] + partial response [PR]) in a sample size of approximately 128 pts) using RECIST v1.1. Subgroup analyses evaluating ORR by prior treatment and disease burden were performed.

Results: A total of 132 pts were treated with abemaciclib monotherapy. Analyses of subgroups based on prior treatment and disease burden are summarized in Table 1.

Conclusions: Abemaciclib monotherapy administered on a continuous schedule has consistent single agent activity across the subgroups analyzed.

Table 1. Objective Response Rate Subgroup Analyses

	Number of Patients	Objective Response (CR + PR) n(%)
ITT	132	26 (19.7)
Number of Prior chemotherapies for metastatic disease		
1	67	14 (20.9)
2	64	11 (17.2)
3	1	1 (100.0)
Taxane use in the metastatic setting		
Yes	91	19 (20.9)
No	41	7 (17.1)
Capecitabine use in the metastatic setting		
Yes	76	16 (21.1)
No	56	10 (17.9)
Number of prior endocrine therapies for metastatic disease		
0	17	3 (17.6)
1	48	9 (18.8)
2 or more	67	14 (20.9)
Fulvestrant use in the metastatic setting		
Yes	67	15 (22.4)
No	65	11 (16.9)

Number of metastatic sites		
1	19	4 (21.1)
2	46	10 (21.7)
3 or more	67	12 (17.9)
Liver metastases		
Yes	93	20 (21.5)
No	39	6 (15.4)

References: 1. Dickler, M. et al. American Society of Clinical Oncology (ASCO), abstract #510 (2016).

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-13

Title: Phase Ib study evaluating the safety and clinical activity of lumretuzumab combined with pertuzumab and paclitaxel in HER2-low metastatic breast cancer

Schneeweiss A, Park-Simon T-W, Albanell J, Lassen U, Cortes J, Dieras V, May M, Schindler C, Marmé F, Cejalvo JM Miguel, Martinez-Garcia M, Gonzalez I, Lopez-Martin J, Welt A, Joly F, Michielin F, Jacob W, Adessi C, Moisan A, Meneses-Lorente G, James I, Ceppi M, Hasmann M, Weisser M and Cervantes A. National Center for Tumor Diseases, University Hospital, Heidelberg, Germany; 2Clinics of Obstetrics and Gynecology, Hannover Medical School, Hannover, Germany; Hospital del Mar, Barcelona, Spain; Rigshospitalet, Copenhagen, Denmark; Vall d'Hebron Institute of Oncology, Barcelona, Spain; Institut Curie, Paris, France; Biomedical Research Institute INCLIVA, University of Valencia, Valencia, Spain; Hospital Universitario 12 de Octubre, Madrid, Spain; West German Cancer Centre, University Hospital Essen, Essen, Germany; Center François Baclesse, Caen, France; Roche Pharmaceutical Research and Early Development, Roche Innovation Center Basel, Basel, Switzerland; Roche Pharmaceutical Research and Early Development, Roche Innovation Center Munich, Penzberg, Germany; Pharma Research and Early Development, Clinical Pharmacology, Roche Innovation Center Welwyn, Welwyn Garden City, United Kingdom and A4P Consulting Ltd, Sandwich, United Kingdom.

Body: Background: Inhibition of HER2 and HER3 heterodimerisation is a novel treatment concept in HER2-"low" expressing breast cancer (BC). Lumretuzumab, a glycoengineered monoclonal anti-HER3 antibody, in combination with pertuzumab has demonstrated synergistic anti-tumor activity in preclinical HER2-low expressing preclinical BC models.

Methods: This open-label, multicenter phase I study selectively enrolled metastatic BC patients (pts) expressing HER3 protein and low levels of HER2 (defined as IHC 1+ and 2+ and ISH-negative) in a formalin-fixed paraffin-embedded pretreatment tumor biopsy sample. Eligible pts were treated with a combination of paclitaxel (PA) qw plus lumretuzumab (L) and pertuzumab (P) q3w in three dose cohorts. The safety, antitumor activity and tumor biomarkers including protein expression (IHC, MS) and mutational data (NGS) in association with clinical activity were evaluated.

Results: Overall, 35 pts were included in this study. The median age was 60 (range: 33 to 77) years. The median number of prior treatments for metastatic disease ranged from 0 to 5 with 23 pts (65.7%) without prior chemotherapy for metastatic disease. Cohort 1 was treated with PA at 80 mg/m², L at 1000 mg and P at 840 mg for Cycle 1 followed by 420 mg for the following cycles. This cohort was stopped after two pts both experienced grade 3 diarrhea within the first treatment cycle which was considered a dose-limiting toxicity (DLT). For Cohort 2 the dose of L was reduced to 500 mg based on PK modelling and simulation data. No DLTs were seen for the first 6 pts. A total of 20 pts were recruited with an objective response rate (ORR) and disease control rate (DCR) of 30% and 75%, respectively, and 56% and 78%, respectively, for 1st-line pts (n=9) in this cohort. Diarrhea (≥G3) and hypokalemia (≥G3) occurred in 50% and 55% of pts, respectively, and all pts experienced chronic diarrhea throughout the course of treatment. For Cohort 3 the dose of L was maintained at 500 mg, PA at 80 mg/m², and P was administered at 420 mg at all cycles. In addition, a prophylactic loperamide regimen was introduced. Altogether, 13 pts - all 1st-line for metastatic disease - were treated. No DLTs were seen for the first 6 pts. Diarrhea (≥G3) and hypokalemia (≥G3) were reduced to 31% and 15%, respectively, but chronic diarrhea was still observed throughout the treatment in all pts. The ORR and DCR were 31% and 77%, respectively. Preliminary mechanistic safety experiments revealed HER2/HER3-dependent chloride channels in the intestine as likely cause of diarrhea. Biomarker data will be presented along with updated clinical and safety data.

Conclusions: The combination of L, P and PA was associated with high rates of persistent diarrhea. Dose modifications and prophylactic anti-diarrheal medication led to significantly reduced diarrhea intensity but did not change the incidence and persistence of diarrhea overall. Despite encouraging clinical activity especially in 1st line pts, the therapeutic window of this combination is too low to warrant further clinical development.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-14

Title: Src homology 2 domain containing transforming protein 1 and steroid receptor coactivator-3 as novel targets for triple-negative breast cancer

Chang Y-F, Wang Y and Greene GL L. The University of Chicago, Chicago, IL.

Body: Triple-negative breast cancer (TNBC) accounts for 15-20% of breast cancer cases and is more prevalent among young women and African American women. Metastasis and chemoresistance remain significant challenges in TNBC. The development of targeted therapies for TNBC may dramatically improve the survival of patients and the quality of life. An adaptor protein, Src Homology 2 Domain Containing Transforming Protein 1 (SHC1) and steroid receptor coactivator-3 (NCOA3) are found to be overexpressed in breast cancers and important in the regulation of tumor progression and metastasis and drug resistance in ER+ breast cancer. The elevated SHC1 or NCOA3 correlates with poor prognosis in breast cancer patients. However, the biological significance of SHC1 and NCOA3 in TNBC is not well known. In this study, we performed siRNA-mediated knockdown to determine the importance of SHC1 and NCOA3 in the cell proliferation and death of TNBC. The effect of siSHC1 or siNCOA3 on the invasion and chemoresistance was also assayed in vitro. We found that although SHC1 and NCOA3 knockdown slightly inhibited the tumor growth of TNBC cells (MDA-MB-231, BT549, and HS578T), siSHC1 + paclitaxel and siNCOA3 + paclitaxel significantly decreased cell proliferation and increased caspase-3/7 activity in vitro, compared to drug alone. In vivo studies using MDA-MB-231 xenografts and a TNBC PDX model also showed that siSHC1 and siNCOA3 significantly augmented paclitaxel-induced tumor shrinkage. In invasion assays, SHC1 and NCOA3 knockdown significantly decreased the invasion of TNBC cells. These results suggest that targeting SHC1 and NCOA3 is worth investigating for the treatment of TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-15

Title: Pre-clinical investigation of estrogen receptor β agonists for the treatment of breast cancer

Samayoa C, Krishnegowda NK K, Vadlamudi RK K and Tekmal RR R. University of Texas Health Science Center at San Antonio, San Antonio, TX.

Body: Breast Cancer is the primary cause of cancer-associated mortality worldwide, and in United States alone, more than 250,000 women are diagnosed every year. Current breast cancer treatment strategies focus on Estrogen Receptor α signaling, given that the majority of cases diagnosed are ER α positive. These treatment strategies include endocrine therapies; such as anti-estrogens or aromatase inhibitors. Although, endocrine therapy has been demonstrated to be successful and effective, therapy resistance commonly arises and results in relapse. While current endocrine therapies focus on ER α signaling, emerging studies highlight the importance of Estrogen Receptor β . Unlike ER α , ER β has been shown to have tumor-suppressive function in various cancers, including breast cancer. Recent studies have identified, synthesized, and tested the clinical safety of ER β -selective agonists. The objective of this study was to investigate the utility of using ER β agonists in the treatment of breast cancer.

To investigate the utility of ER β agonists in the treatment of breast cancer, we used in-vitro and in-vivo pre-clinical models systems. Our results demonstrated that treatment with ER β agonists, S-Equol and LY500307, was able to inhibit the short-term and long-term growth of both endocrine therapy sensitive and resistant breast cancer cells. Progression through the cell cycle, cell migration and cell invasion was also abrogated upon treatment. In-vivo, our syngeneic tumor mouse model demonstrates a decline in tumor growth rate after treating with a combination of letrozole and ER β agonist. Gene expression array analysis reveal that treatment with ER β agonist elicits changes in key signaling molecules involved in cell death and cell cycle pathways. In Letrozole resistant cells, Letrozole treatment had not effect on gene expression, while LY500307 treatment resulted in the modulation of 780 genes. Interestingly, combining Letrozole with LY500307 resulted in the modulation of 966 genes, of which 417 were unique to the combination treatment. Our studies suggest that activation of ER β signaling is a valuable strategy in the treatment of breast cancer, even in cases which have developed resistance to current endocrine therapies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-16

Title: PYTHIA: A phase II study of palbociclib plus fulvestrant versus placebo plus fulvestrant for pretreated patients with ER+/HER2- metastatic breast cancer

Zardavas D, Regan M, Maibach R, Ruepp B, Hiltbrunner A, Blacher L, Goulioti T, Gelber R, Flamen P, Piccart M and Malorni L. Breast International Group, Brussels, Belgium; International Breast Cancer Study Group, Bern, Switzerland; Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA; Frontier Science and Technology Research Foundation, Amherst, NY; Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium and Hospital of Prato, Azienda USL Toscana Centro, Prato, Italy.

Body: Background: Palbociclib (PD 0332991) is an orally bioavailable, potent, and selective inhibitor of CDK4/6, blocking cell cycle progression from G1 into S phase. Preclinical data indicate that palbociclib has enhanced activity against luminal ER+ human breast cancer (BC) cell lines in vitro, showing synergy with endocrine treatment. Recently, randomized clinical trials showed significant PFS prolongation in patients with newly diagnosed and pretreated metastatic luminal BC, when palbociclib was combined with letrozole and fulvestrant respectively (PALOMA-1/2 and -3 trials). However, predictive biomarkers for patient selection to receive palbociclib plus endocrine treatment are still missing.

Study design: PYTHIA is a phase II, two-arm, randomized (3:1), double-blind, multicenter, study of fulvestrant and palbociclib versus fulvestrant and placebo in postmenopausal women with ER+/HER2-, advanced BC, progressed after prior endocrine therapy (1st or 2nd line). Of note, only patients successfully enrolled in the AURORA program (NCT02102165), a longitudinal cohort study incorporating extensive molecular characterization of matched primary and metastatic BC samples are eligible. The primary endpoint is PFS, based on local assessment of response using RECIST 1.1. Secondary endpoints are safety and tolerability, as well as disease control rate. PYTHIA incorporates several correlative objectives, with the exploration of the potential predictive value of: i) mutations and copy number aberrations in a panel of cancer-related genes in both primary and metastatic tumor lesions, ii) gene signatures inferred by RNA sequencing, iii) early FDG-PET/CT assessment performed by two blinded, independent experts, for a subset of 30 patients, at baseline and Day 28, and iv) a serum thymidine kinase-1 (TK1) assay, performed at baseline and after Cycle 1.

Statistical methods:

The sample size was determined in consideration of the primary and correlative objectives. The median PFS of patients treated with fulvestrant alone is assumed to be 6 months. When 92 events are observed, there is 75% power to detect an improvement in median PFS from 6 to 10.5 months (43% reduction in hazard, HR=0.57; one-sided $\alpha=0.05$) with palbociclib plus fulvestrant, and 52% power to detect a 50% improvement in median PFS to 9 months (33% reduction in hazard, HR=0.67).

Target accrual: Enrollment opened in May 2016, with the target-recruitment being 120 patients at 21 sites in Belgium, Italy and the UK.

Contact information:

Reference Study ID Numbers: IBCSG 53-14 / BIG 14-04.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-17

Title: Mutations in PIK3R1 activate multiple pathways in breast cancer

Abukhdeir AM M, Turturro SB B, Najor MS S, Brar SS S and Cobleigh MA A. Rush University Medical Center, Chicago, IL.

Body: It has been estimated that by the end of this year, 270,000 American women will be newly diagnosed with breast cancer, while 40,000 women that already have breast cancer will succumb to the disease. Considerable attention has been given to the PI3K signaling cascade following the discovery that *PIK3CA* is the most frequently mutated oncogene in breast cancer. However, few studies have explored the function of PIK3R1, which is the regulatory domain of the PI3K complex, despite being mutated in ~3% of all breast cancers.

Several studies have demonstrated that expression of *PIK3R1* is downregulated in human cancers. Decreased expression of the PIK3R1 protein leads to tumor formation, suggesting its role as a tumor suppressor gene and a potential prognostic marker in breast cancer. However, *PIK3R1* is a gene with little pre-clinical evidence to recommend experimental therapies. Despite this lack of evidence, commercial services that perform molecular analyses of tumors suggest the use of an mTOR inhibitor for patients whose breast cancers carry mutant *PIK3R1*.

In order to determine if mTOR inhibitors were indeed effective in mutant *PIK3R1* tumors, we created and characterized a model for mutant *PIK3R1* in the non-tumorigenic, human breast epithelial cell line, MCF-10A. Surprisingly, we observed that mTOR inhibitors were ineffective in these cells. However, in searching for other classes of small molecule inhibitors that were effective, we observed that mutations in *PIK3R1* sensitized cells to MAPK inhibitors.

Herein, we present the first evidence for the use of targeted therapies in breast cancers carrying mutant *PIK3R1*. We provide evidence against the use of mTOR inhibitors and provide a rationale for the use of MAPK inhibitors.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-11-18

Title: A novel subgroup of estrogen receptor positive breast cancer may benefit from super-enhancer guided patient selection for retinoic acid receptor α agonist treatment

McKeown MR R, Fiore C, Lee E, Eaton ML L, Orlando D, Guenther MG G, Collins C, Chen MW Wei, Fritz CC C and di Tomaso E. Syros Pharmaceuticals, Cambridge, MA.

Body: Endocrine-resistance remains a major challenge for treatment of breast cancer. Multiple mechanisms for endocrine resistance have been proposed, including altered expression of ER co-regulators such as Retinoic Acid Receptor Alpha (RAR α). Furthermore, crosstalk between estradiol and RA signaling is known and upregulation of RAR α has been observed in tamoxifen resistance. We propose a novel treatment paradigm for a newly-defined subset of HR+ patients based on our discovery of a super-enhancer (SE) associated with the *RARA* locus. SEs are large, highly active chromatin regions that pinpoint cancer vulnerabilities. The *RARA* SE-identified vulnerability can be targeted using the potent, selective, and metabolically stable RAR α agonist SY-1425 (tamibarotene). SY-1425 is approved in Japan to treat Acute Promyelocytic Leukemia, has a well-established efficacy and safety profile, and may enhance response to hormonal therapy (HT) in this newly-defined subset of HR+ patients potentially delaying the need for alternate treatment.

Tumor samples from 42 breast cancer patients were analyzed across a range of molecular subtypes. We identified an SE linked to the *RARA* gene in 54.5% of the hormone positive patient samples. *RARA* SEs predicted sensitivity to SY-1425 in 12 breast cancer cell lines confirming their functional role, and showed a correlation with *RARA* gene expression. A panel of 37 breast cancer cell lines was tested for SY-1425 anti-proliferative activity and gene expression levels, and identified *RARA* as the single best predictor of response. Proliferation of RARA-high cells was inhibited by SY-1425 with low nanomolar EC50s. Transcriptional profiling was performed on 4 HR+ and 3 HER2+/HR- breast cancer cell lines and analyzed by GSEA to examine the molecular response to SY-1425. Signatures for growth including E2F, MYC, DNA replication, and cell cycle were significantly downregulated while retinol metabolism and luminal signaling were upregulated. Estrogen signaling was also significantly altered by SY-1425, supporting known crosstalk between RAR α and ER. Consistent with differentiation, CYP26A1 and VE-Cadherin were induced and Actin and Ki67 were diminished at relevant concentrations of SY-1425 and could serve as pharmacodynamic markers of response.

To test responses to SY-1425 *in vivo*, two cell line-derived models and two patient-derived breast cancer models (one RARA-high, and one RARA-low each) were treated with SY-1425. SY-1425 inhibited tumor growth in the RARA-high models, but not the RARA-low models (43% versus 0% TGI). Consistent with the observed changes in transcription, SY-1425 in combination with tamoxifen synergistically inhibited proliferation of RARA-high breast cancer cell lines.

Although a few clinical studies have investigated the use of ATRA in HR+ breast cancer without success, our results suggest that patient selection based on the *RARA* SE may predict which HR+ breast cancer patients could derive benefit by adding an RAR α agonist to HT. The potential to prolong or increase the clinical effect of anti-estrogen therapy with SY-1425, which has improved potency, selectivity, and PK stability versus ATRA, would be an attractive strategy to explore.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-01

Title: Phase II study of taselisib (GDC-0032) plus fulvestrant in HER2-negative, hormone receptor-positive advanced breast cancer: Analysis by *PIK3CA* and *ESR1* mutation status from circulating tumor DNA

Dickler MN N, Saura C, Oliveira M, Richards DA A, Krop IE E, Cervantes A, Stout TJ J, Jin H, Savage HM M, Wilson TR R and Baselga J. Memorial Sloan Kettering Cancer Center, Memorial Hospital, New York, NY; Vall d'Hebron University Hospital, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; US Oncology Research, Woodlands, TX; Dana-Farber Cancer Institute, Boston, MA; Institute of Health Research INCLIVA, University of Valencia, Valencia, Spain and Genentech Inc., South San Francisco, CA.

Body: Background:

The phosphatidylinositol 3-kinase (PI3K) pathway is frequently dysregulated in hormone receptor (HR)-positive breast cancer (BC), with activating mutations of *PIK3CA* detected in ~35–45% of patients (pts). Acquired mutations in the *ESR1* gene, which encodes estrogen receptor α , may be associated with resistance to aromatase inhibitor (AI) therapy. Taselisib is a potent and selective PI3K inhibitor, with greater selectivity against mutant PI3K α isoforms than wild-type (WT) via a unique mechanism. In phase I studies, taselisib plus fulvestrant had clinical activity and manageable tolerability in pts with HR-positive BC. We report exploratory analyses of *PIK3CA* and *ESR1* from circulating tumor DNA (ctDNA).

Methods:

In this phase II, open-label, single-arm study (PMT4979g; NCT01296555), pts were postmenopausal with HER2-negative, HR-positive locally advanced or metastatic BC and progression or non-response to ≥ 1 prior endocrine therapy in the adjuvant or metastatic setting. Pts received taselisib (6 mg capsule orally, daily) plus fulvestrant (500 mg intramuscular on Days 1 and 15 of Cycle 1, then Day 1 of each 28-day cycle) until disease progression or unacceptable toxicity. *PIK3CA*-mutation testing on archival tumor tissue used the cobas[®] *PIK3CA* Mutation Test. The Sysmex Inostics' BEAMing Digital PCR platform was used for ctDNA analysis of *ESR1* and *PIK3CA* mutations (pre-dose on Cycle 1, Day 1). Primary endpoints were objective response rate (ORR) and clinical benefit rate (CBR) in all pts and those with *PIK3CA* mutations. ORR was confirmed complete response (cCR) and confirmed partial response (cPR). CBR was cCR, cPR, or stable disease for ≥ 6 months. Secondary endpoints included safety, efficacy, pharmacokinetics, and exploratory biomarker analysis.

Results:

60 pts were enrolled. Median age was 61.5 years (range 31–82). In the metastatic setting, pts had received prior chemotherapy (21.7%) and prior hormonal therapy (50.0%). 86.7% of pts had received prior treatment with an AI. 45 pts had *PIK3CA* mutation status from archival tumor tissue and ctDNA testing; concordance was 86.7% (39/45). ctDNA analysis, vs archival tumor tissue testing, identified 4 pts and 9 pts with *PIK3CA* mutations from pts with WT and unknown *PIK3CA* mutation status, respectively. Based on ctDNA analysis (N=60), 13 pts (21.7%) had mutations in both *ESR1* and *PIK3CA*, 21 pts (35.0%) were 'mutation not detected' (MND) for both genes, 8 (13.3%) had *ESR1* mutations and *PIK3CA* MND, and 18 (30.0%) had *ESR1* MND and *PIK3CA* mutations.

In pts with measurable disease at baseline, confirmed responses (all partial) were: *PIK3CA* mutation, 38.1% (8/21); *PIK3CA* MND, 8.7% (2/23); all pts, 22.7% (10/44). CBRs were: *PIK3CA* mutation, 42.9%; *PIK3CA* MND, 17.4%; all pts, 29.5%. ORR and CBR from ctDNA analyses were similar to archival tumor tissue data.

Conclusions:

ctDNA analysis identified *PIK3CA* mutations in pts with previously unknown or WT mutation status from archival tumor tissue; ORR and CBR were similar to those from archival tumor tissue suggesting that *PIK3CA* mutation testing from ctDNA may be used as a surrogate when tissue is unavailable. 21.7% of pts had mutations in both *ESR1* and *PIK3CA*.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-02

Title: SYD985, a novel anti-HER2 ADC, shows promising activity in patients with HER2-positive and HER2-negative metastatic breast cancer

Aftimos PG G, van Herpen CM M, Mommers EC C, Koper NP P, Goedings P, Oesterholt M, Awada A, Desar IM M, Lim J, Dean E, Rolfo C, Macpherson I and Banerji U. Institut Jules Bordet - Université Libre de Bruxelles, Brussels, Belgium; Radboud University Medical Center, Nijmegen, Netherlands; Synthon Biopharmaceuticals BV, Nijmegen, Netherlands; The Institute of Cancer Research and The Royal Marsden, London, United Kingdom; The Christie NHS Foundation Trust, Manchester, United Kingdom; University Hospital Antwerp, Edegem, Belgium and Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom.

Body: Background:

SYD985 is a HER2-targeting antibody-drug conjugate (ADC) based on trastuzumab and a cleavable linker-duocarmycin (vc-seco-DUBA) payload. Following proteolytic cleavage the synthetic duocarmycin prodrug is activated, binds to the minor groove of DNA, and subsequently causes irreversible DNA-alkylation. SYD985 has demonstrated unprecedented anti-tumor activity in preclinical breast cancer models with high (HER2 3+) or moderate/low expression of HER2 (HER2 2+ and HER2 1+). Clinical data of patients with HER2-positive and HER2-negative breast cancer treated with SYD985 in an ongoing first-in-human phase I trial (NCT02277717) in patients with locally advanced or metastatic solid tumors are presented.

Trial design:

Main inclusion criteria are ECOG performance status 0-1, left ventricular ejection fraction $\geq 55\%$, and adequate organ function. Patients are treated with SYD985 every three weeks until tumor progression or unacceptable toxicity. Thirty nine patients, including 26 breast cancer patients, were enrolled in the dose-escalation part and treated with doses varying from 0.3 mg/kg to 2.4 mg/kg SYD985. Patients enrolling in the first expanded cohort will be treated with 1.2 mg/kg SYD985. In this cohort a total of 48 patients with HER2-positive breast cancer (IHC 3+ or ISH positive) will be enrolled from May 2016 onwards. HER2 status was determined locally in the dose-escalation part but will be done centrally in the expanded cohort part of the trial. Tumor evaluation (RECIST 1.1) is performed every 6 weeks.

Results:

Enrolled breast cancer patients were heavily pretreated with a median of 7 systemic therapies. All patients with HER2-positive breast cancer were previously treated with trastuzumab and ado-trastuzumab emtansine (T-DM1). As of 16 May 2016, tumor evaluation data were available for 19 of the 26 enrolled breast cancer patients. In total, 8 partial responses were observed of which 6 were confirmed by a second CT-scan. The number of cycles administered ranged from 1 to 11. Ten of the 26 patients are still on treatment.

HER2 status	N Evaluable	Best overall response			ORR All doses	ORR Doses ≥ 1.2 mg/kg
		PR	SD	PD		
HER2-positive (all T-DM1 pretreated)	14	5	8	1	36%	42%
HER2-negative (IHC 1+/2+ and ISH neg)	5	3	1	1	60%	75%
Overall	19	8	9	2	42%	50%

One dose-limiting toxicity occurred at 2.4 mg/kg SYD985, i.e. pneumonitis (grade 5). Overall, SYD985 is well tolerated up to doses of 1.8 mg/kg every 3 weeks. The most frequently reported drug-related AEs were conjunctivitis, stomatitis, fatigue, and decreased appetite. The majority of drug-related AEs were of mild or moderate intensity.

Conclusion:

SYD985 shows promising efficacy in both HER2-positive and HER2-negative metastatic breast cancer patients with an acceptable safety profile. SYD985 may offer a new targeted treatment option for patients who have become refractory to the available HER2-targeting therapies, and potentially for breast cancer patients who are not indicated for HER2-targeting therapies. Updated data, including additional results of up to 48 HER2-positive breast cancer patients, will be presented during the meeting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-03

Title: A phase I study of AZD9496, a novel oral, selective estrogen receptor degrader (SERD) in women with estrogen receptor positive, HER-2 negative advanced breast cancer (ABC)

Hamilton E, Patel M, Armstrong A, Baird R, Jhaveri K, Hoch M, Morgan S, Dowdall T, Schiavon G, Klinowska T, Weir H, Bujac S, Nash T and Im S-A. Sarah Cannon Research Institute/Tennessee Oncology; Sarah Cannon Research Institute/Florida Cancer Specialists; The Christie NHS Foundation Trust and the University of Manchester, United Kingdom; Cambridge Cancer Centre, United Kingdom; Memorial Sloan Kettering Cancer Center, New York; AstraZeneca, Cambridge, United Kingdom and Seoul National University Hospital, Korea.

Body: Background AZD9496 is a potent orally bioavailable ER antagonist and degrader that has shown antitumor efficacy in a range of preclinical xenograft models including ESR1 wild-type tamoxifen-resistant and long term estrogen deprived models and an ESR1 mutant model.

Methods This is a phase I, open label global multicenter study in women with ER+ HER2-ve BC either metastatic or locoregionally recurrent, not amenable to treatment with curative intent. Patients are post-menopausal, or pre-menopausal women receiving LHRH agonist therapy, with disease progression after ≥ 6 months endocrine therapy for ER+ BC (no limit on number of prior endocrine therapies; ≤ 2 prior chemotherapies in advanced setting). The primary objective is to determine the safety and tolerability of AZD9496. Cohorts of 3-6 patients received daily oral therapy and dose limiting toxicities (DLTs) occurring in cycle 1 (28 days) were assessed. Patients are dosed until MTD (defined as $\leq 1/6$ patients with a DLT) or maximum feasible dose (MFD) is reached. Key secondary objectives include determination of single and multiple dose pharmacokinetics (PK), and preliminary antitumor efficacy. ER target modulation by protein and gene expression is evaluated in circulating tumor cells and paired tumor biopsies.

In addition to the dose escalation phase, expansion cohort(s) in patients with or without ESR1 mutations can be enrolled to examine the safety, tolerability, PK and biological activity of AZD9496 further.

Results Preliminary data as of 30th April 2016: 45 patients (median age 62 (range 41-83); 38 post-menopausal, 7 pre/perimenopausal; visceral metastases 76%, prior fulvestrant 25/45) received AZD9496 in 7 dose escalation cohorts: 20mg QD n=4, 40mg BID n=6, 80mg BID n=5, 150mg BID n=6, 250mg BID n=6, 400mg BID n=6, 600mg BID n=6 and also a 250mg BID expansion cohort n=6. The majority of adverse events (AEs) were grade 1 or 2; the most common treatment-related AEs ($\geq 10\%$) have been diarrhoea (33%), fatigue (27%), nausea (22%), upper abdominal pain (13%) and increased liver function tests (13%). Six patients had treatment-related grade 3 AEs, 5 of which were manageable with dose interruption +/- dose reduction. Specifically, three had DLTs: grade 3 increased AST/ALT/GGT-150mg BID, serious adverse reaction (SAR) leading to withdrawal; grade 3 diarrhoea and grade 3 increased AST/ALT/GGT-400mg BID, SAR, manageable with dose reductions; grade 3 diarrhoea 600mg BID, manageable with dose reduction. The MTD/MFD has not been reached.

Following the first dose up to 400mg the AZD9496 exposure increased in reasonable proportion to increasing dose. At 600mg a more than dose-proportional increase in exposure was observed. Evidence of reduced ER and Ki67 has been observed in on-study biopsies at 150mg BID and above. 10 subjects received treatment for >3-<6 months (5 ongoing, 5 discontinued), 4 subjects >6-<12 months (3 ongoing, 1 discontinued), 3 subjects ≥ 1 year (2 ongoing, 1 discontinued).

Conclusions AZD9496 has a tolerable safety profile, evidence of PD biomarker modulation and prolonged stabilisation of disease in women with heavily pre-treated ER+ve ABC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-04

Title: Phase 1 study of the antibody-drug conjugate (ADC) SGN-LIV1A in patients with heavily pretreated metastatic breast cancer

Forero-Torres A, Modi S, Specht J, Miller K, Weise A, Burris III H, Liu M, Krop I, Pusztai L, Kostic A, Li M and Mita M. University of Alabama at Birmingham, Birmingham, AL; Memorial Sloan Kettering Cancer Center, New York, NY; Seattle Cancer Care Alliance, University of Washington, Seattle, WA; Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN; Karmanos Cancer Institute, Detroit, MI; Sarah Cannon Research Institute/Tennessee Oncology, Nashville, TN; Mayo Clinic, Rochester, MN; Dana-Farber Cancer Institute, Boston, MA; Yale University School of Medicine, New Haven, CT; Seattle Genetics, Inc., Bothell, WA and Cedars-Sinai Medical Center, Los Angeles, CA.

Body: Background

LIV-1, a transmembrane protein and downstream target of STAT3, is highly expressed in breast cancer cells. It is associated with lymph node involvement and metastatic progression. SGN-LIV1A is an anti-LIV-1 antibody conjugated via a protease-cleavable linker to monomethyl auristatin E (MMAE). Upon binding to cell-surface LIV-1, SGN-LIV1A is internalized and releases MMAE, which binds to tubulin and induces G2/M arrest and apoptosis.

Methods

This is an ongoing, phase 1 dose-escalation study evaluating safety, tolerability, pharmacokinetics, and antitumor activity of SGN-LIV1A (q3 wks IV) in women with LIV-1-positive, unresectable, locally advanced or metastatic breast cancer (LA/MBC) (NCT01969643). Patients (pts) with measurable disease and ≥ 2 prior cytotoxic regimens for LA/MBC were eligible. Pts with \geq Grade 2 neuropathy were excluded. Response was assessed per RECIST v1.1; pts with stable disease (SD) or better could continue treatment until disease progression or intolerable toxicity. At completion of dose escalation in hormone receptor-positive/HER2-negative (HR+/HER2-) and triple-negative (TN) pts, expansion cohorts were opened to further evaluate safety and antitumor activity of monotherapy in TN pts and combination therapy with trastuzumab (Tz) in HER2-positive (HER2+) pts. Pre- and post-treatment tumor biopsies were done to evaluate LIV-1 expression and other correlative endpoints.

Results

To date, 39 pts (18 HR+/HER2-, 21 TN) have received a median of 3 cycles (range, 1–10) of SGN-LIV1A monotherapy at doses of 0.5–2.8 mg/kg. Median age was 57 yrs (range, 33–79). At baseline, pts had a median of 4 prior cytotoxic regimens for LA/MBC (range, 2–8); 36 had visceral disease and 25 had bone involvement. No dose-limiting toxicities (DLT) occurred in 19 DLT-evaluable pts; maximum tolerated dose was not exceeded at 2.8 mg/kg. Treatment-emergent adverse events (AEs) reported in $\geq 30\%$ of pts were: fatigue (64%), nausea (54%), alopecia (46%), decreased appetite (41%), constipation (39%), neutropenia (33%), and vomiting (31%). Peripheral neuropathy was reported in 9 pts (23%). Most AEs were Grade 1/2, except neutropenia (all \geq Grade 3). Four pts discontinued treatment due to AEs (acute respiratory distress syndrome, nausea, pneumonia, tachycardia). In dose escalation, modest activity was observed in 17 efficacy evaluable (EE) HR+/HER2- pts, with a disease control rate (DCR) of 59% (10 SD), including 1 pt with SD ≥ 24 wks. Among the 17 EE TN pts (dose escalation plus cohort expansion), the overall response rate (ORR) was 41% (7 PR), DCR was 82% (7 PR, 7 SD) and clinical benefit rate (CBR=OR+SD ≥ 24 wks) was 53% (9 pts). For TN pts, median PFS was 17.1 wks (95% CI: 6.0, 18.4); 6 pts remain on treatment. Of 281 MBC tumor samples evaluated for LIV-1, 93% were positive; 81% had moderate-to-high expression (H-score ≥ 100).

Conclusions

LIV-1 is expressed in almost all MBC tumors. SGN-LIV1A monotherapy has been generally well tolerated and shown encouraging antitumor activity in heavily pretreated TN MBC, with a PR rate of 41% and a CBR at ≥ 24 wks of 53%. Response duration data continue to evolve. Enrollment continues in the TN monotherapy expansion cohort and the HER2+ combination cohort with Tz.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-05

Title: Targeting estrogen receptor mutations for treatment of endocrine therapy resistance in breast cancer

Alluri PG G, Larios JM M, Malik R, Hayes DF F, Speers CW W, Rae JM M and Chinnaiyan AM M. University of Michigan Medical School, Ann Arbor, MI.

Body: Objectives:

Breast cancer is the most commonly diagnosed non-cutaneous cancer and the second leading cause of cancer-related deaths (> 40,000 estimated deaths in 2015) among women in the United States. Approximately 70% of all breast cancers express the Estrogen Receptor (ER) and inhibition of the ER signaling remains the mainstay of systemic therapy in such cancers. However, acquired endocrine therapy resistance is a major obstacle to effective treatment of a significant proportion of ER-positive breast cancers. Our group and others have recently reported mutations in the gene encoding the Estrogen Receptor (*ESR1*) in metastatic breast cancer patients. Preliminary studies have suggested that *ESR1* mutations confer endocrine therapy resistance in ER-positive breast cancers. Recent studies have shown that bromodomain and extraterminal (BET) family of proteins act downstream of ER activation and regulate the transcriptional function of ER. We hypothesized that BET inhibition serves as a novel strategy for targeting *ESR1* mutations and represents an attractive approach for treatment of endocrine therapy resistance.

Methods: *In vitro* models of endocrine therapy resistance in breast cancer were developed by ectopic expression of two of the most commonly found *ESR1* mutations (Y537S and D538G) in ER-positive cell lines, MCF-7 and T47D. Cell growth assays in estradiol-free conditions were used to assess growth of these cells in the presence of tamoxifen and BET inhibitor, OTX015.

Results: MCF-7 and T47D cells expressing mutant *ESR1*s show significant growth advantage when compared to the isogenic cells expressing the wild type *ESR1* in estradiol-free conditions (which mimic the estradiol-free state of post-menopausal women treated with aromatase inhibitors). Cells expressing the *ESR1* mutations showed remarkable resistance to growth inhibition by tamoxifen while cells expressing the wild type *ESR1* were highly sensitive. BET inhibitor, OTX015, was highly effective in inhibiting the growth of breast cancer cells expressing *ESR1* mutations. OTX015 was also highly effective in down-regulating canonical ER-responsive genes such as Gene Regulated by Estrogen in Breast Cancer 1 (*GREB1*) in cells harboring *ESR1* mutations while tamoxifen only showed marginal effectiveness.

Conclusion: *ESR1* mutations confer endocrine therapy resistance in breast cancer. BET inhibitor OTX015 targets the transcriptional function of mutant ER proteins and is effective in reversing endocrine therapy resistance due to *ESR1* mutations in *in vitro* models of breast cancer. Further validation of our findings in *in vivo* models may provide impetus for clinical evaluation for BET inhibitors for treatment of endocrine therapy resistance due to *ESR1* mutations.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-06

Title: Nonsteroidal, tissue selective androgen receptor modulator (SARM), enobosarm, reduces growth of androgen receptor-positive breast cancer in patient-derived preclinical models

Narayanan R, Ponnusamy S, Fan M, Yang CH, Grimes BL L, Fleming MD D, Pritchard EF F, Berry MP P, Oswaks RM M, Fine RE E, Loiseau J-C, Schwartzberg LS S and Pfeffer LM M. University of Tennessee Health Science Center, Memphis, TN and West Cancer Center, Memphis, TN.

Body: Introduction: In breast cancer the androgen receptor (AR) is the most abundantly expressed steroid receptor with 75-95% of estrogen receptor (ER)-positive and 40-70% of ER-negative breast cancers expressing the AR. Historically, advanced breast cancer has been treated with androgens, resulting in significant clinical response. However, the use of steroidal androgens fell from favor as a result of their virilizing side effects. Nonsteroidal, tissue selective androgen receptor modulators (SARMs) will provide a novel targeted approach to exploit the therapeutic benefits of androgens in breast cancer.

Aims: To test the effects of enobosarm (a first-in-class SARM) and enzalutamide (AR antagonist) on the growth of patient-derived breast cancer xenografts (PDX) and to discern the mechanism of action of AR-targeted therapies in AR-positive breast cancer.

Materials and Methods: AR-positive PDXs with varying receptor expression (ER, progesterone receptor (PR), and HER2) were implanted in immunocompromised mice. Mice carrying PDXs were treated with vehicle, 10 mg/kg/day (mpk) enobosarm (GTx, Inc., Memphis, TN), or 20 mpk enzalutamide (Medivation Inc.), orally. Tumor volume was measured twice or thrice weekly. Tumors that received enobosarm were further analyzed to determine the mechanism of action.

Results: Enobosarm significantly ($p < 0.01$) inhibited the growth of ER-, PR-, and HER2- positive HCl-7 and ER- and PR- negative and HER2-positive HCl-12 PDX. While enobosarm inhibited the growth of HCl-12 by ~80% and HCl-7 by ~60%, enzalutamide failed to inhibit the growth of the HCl-7 PDX. In contrast, neither enobosarm nor enzalutamide inhibited the growth of ER- and PR-negative and HER2-positive HCl-9 PDX, consistent with the heterogeneity of AR-positive breast cancers. Growth of two triple-negative breast cancer (TNBC) PDXs were inhibited by 30-40% by enobosarm, but not by enzalutamide. These results were reproduced in xenografts developed with breast cancer cell lines, MCF-7 and MDA-MB-231 expressing the AR. Gene expression studies conducted with the HCl-12 tumors indicated that enobosarm inhibited the expression of various proliferative genes (MUC2, IL10RA, IGSF1, SLC6A4, and others) and increased the expression of growth inhibitory genes (CYP4F8, MYBPC1, and others). Ingenuity pathway analysis demonstrated that enobosarm inhibited genes that are downstream of HER2 signaling. Interestingly, miR-21-3p, which has been implicated in chemo-resistance, was consistently expressed at approximately 10-50-fold higher than miR-21-5p in PDXs. This imbalance was partially reversed by enobosarm.

Conclusion: These results indicate that AR-positive breast cancers are highly heterogeneous and that enobosarm has promise as novel targeted therapy to treat AR-positive breast cancer. Enobosarm is currently in phase II clinical trial in both ER-positive breast cancer and in TNBC patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-07

Title: Neutralization of BCL2/XL enhances the cytotoxicity of T-DM1 in vivo

Zoeller JJ J, Dillon DA A, Bronson RT T, Sampath D, Levenson J and Brugge JS S. Harvard Medical School, Boston, MA; Brigham & Women's Hospital, Boston, MA; Genentech, San Francisco, CA and AbbVie, Chicago, IL.

Body: One of the most recent advances in the treatment of HER2+ breast cancer is the development of the antibody-drug conjugate, T-DM1. T-DM1 has proven clinical benefits for patients with advanced and/or metastatic breast cancer who have progressed on prior HER2-targeted therapies. However, T-DM1 resistance ultimately occurs and represents a major obstacle in the effective treatment of this disease. We previously identified BCL2 upregulation as a critical component of the adaptive response to inhibition of PI3K/mTOR or HER2, and thus examined whether BCL2/XL combinatorial strategies could improve the initial efficacy of T-DM1.

We have found that combined inhibition of BCL2/XL plus T-DM1 significantly enhances the cytotoxicity of T-DM1 in vivo. The effectiveness of T-DM1 plus BCL2/XL inhibition was evaluated in two patient-derived xenograft (PDX) models, both established from advanced and treatment resistant HER2+ patient pleural effusions (PDX8;12). Animals were randomized into one of four groups: T-DM1, ABT-263, T-DM1 + ABT-263 or vehicles. To minimize thrombocytopenia induced by ABT-263, we included a fifth group that received pulsed treatment of ABT-263 + T-DM1. Notably, unlike continuous treatment, pulsed administration of ABT-263 reduced weight loss to vehicle levels and allowed recovery of platelets. Our results after a 14d treatment period indicate that combined treatment with T-DM1 + ABT-263, the dual BCL2/XL inhibitor, confers an exceptional tumor cell cytotoxic advantage characterized by widespread elimination of the tumor cells.

To quantitate pathological responses in vivo and to incorporate a tumor size measurement, we adapted and applied a Residual Tumor Burden (RTB) score system. We estimate the residual tumor area and viable tumor cell content and calculate an RTB score to summarize these two measurements. Supportive of our H&E observations, the RTB scores highlight the dramatic effectiveness of combination treatment in PDX8 & 12. We have subsequently developed a modified treatment plan that maximizes treatment effectiveness and minimizes side effects. We have also tested this drug combination in two additional PDX models of HER2+ breast cancer.

We also characterized the residual tumor cells that escape combination treatment. Some of the residual tumor nests in the PDX8 tumor model were encapsulated within p63+ intraductal-like structures. These observations recapitulate complete pathological responses with residual DCIS as observed in patient tumors. In addition, we found that T-DM1 either alone or in combination with ABT-263 down regulates HER2 protein levels in PDX8 & 12 tumors. The loss of target expression could, in part, account for a sub-population of the residual cells. Despite initial classification as HER2+ and significant responses to combination treatment, clinical assessment of HER2 status indicated that the PDX8 & 12 xenografts used in our study were FISH- and IHC 0-1+. These results provide strong motivation to extend pre-clinical investigations of this drug combination beyond HER2/FISH+ disease. The dramatic improvement in tumor regression observed in these preclinical studies, together with the safety benefits of ABT-263 modified dosing, provides substantial rationale for the clinical investigation of this drug combination.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-08

Title: Phase I study of low dose oral cyclophosphamide (C) plus the poly-ADP-ribose- polymerase (PARP) inhibitor veliparib (V) in women with HER2/neu-negative inoperable locally advanced/metastatic breast cancer (MBC): NCI P8853

Anampa JD D, Patel M, Pellegrino C, Fehn K, Makower D, Oh S-y, Noah K, Chen A, Sparano JA A and Andreopoulou E. Montefiore Medical Center/ Albert Einstein College of Medicine, Bronx, NY; CTEP/NIH, Bethesda, MD and Weill Cornell Breast Center/ New York Presbyterian Hospital- Weill Cornell Medicine, NYC, NY.

Body: BACKGROUND: PARP, an essential nuclear enzyme, is involved in the recognition of DNA damage and facilitation of DNA base-excision repair (BER). PARP inhibition sensitizes tumor cells to cytotoxic agents which induce DNA damage, including C. Metronomic dosing of C may optimize potential for synergy with PARP inhibitors, and also inhibits angiogenesis (*Kerbel et al, Nat Rev Cancer, 4:423-36, 2004*) and may enhance anti-tumor immunity (Ghiringhelli et al. *Cancer Immunol Immunother* 56:641–648, 2007) V is an oral small molecule inhibitor of PARP which potentiates the antineoplastic activity of DNA damaging agents such as C in MX-1 breast xenograft model (*Donawho et al Clin Cancer Res 13:2728-37, 2007*). We performed a phase I trial of metronomic dose oral C plus V in patients with MBC.

METHODS: The primary objective was to determine the safety and identify the recommended phase II dose (RPTD) of the combination of low-dose oral C once daily in combination with V (100, 200, 300 mg) administered BID for 21 days using a standard 3+3 design. Eligibility included HER2/neu negative MBC, ECOG PS 0-1, and at least 1 prior chemotherapy regimen for MBC. Dose limiting toxicity (DLT) was defined as any Grade 3 non-hematological toxicity or Grade 4 thrombocytopenia/neutropenia occurring during cycle 1. After the RPTD of V was shown to be 200 mg BID with C 50 mg daily, the trial was amended to increase the C dose to 75, 100 and then 125 mg daily until hematologic toxicity was dose-limiting.

RESULTS: 31 patients were enrolled, 19 treated with 50 mg of C and 12 treated at higher doses (75-125 mg), with V doses ranging from 50 mg-300 mg BID (see table);5 patients with not evaluable due to rapid disease progression (N=2), non-compliance (N=2), or tumor pain that was not a DLT (N=1). Median age was 52 years (28-72 years), 14 (45 %) had triple negative disease, all had at least 1 prior chemotherapy regimen for metastasis (median 2, range 1-8), and, 7 had germline BRCA mutations, (3 BRCA1 and 4 BRCA2). When combined with 50 mg C daily, RPTD of V was 200 mg PO BID, with nausea being DLT at 300 mg BID. DLT was not observed in any of the 9 additional patients. The median number of cycles given was 3 (range 1-14). Clinical benefit (response or stable disease for at least 24 weeks) occurred in 3/7 (43%), 1/3 (33%) and 1/16(6%) for BRCA mutated, BRCA negative and BRCA unknown, respectively. Median progression-free survival was 4.3 months (1.2-10.9 months) for BRCA mutated patients and 2 months (0.7-10 months) for non-mutated.

CONCLUSIONS: The combination of oral continuous dosing of V (200 mg PO BID) with metronomic C (50, 75, 100 and 125 mg daily) is well tolerated and shows antitumor activity in patients with BRCA mutation associated MBC. The RPTD is C 125 mg daily plus V 200 mg BID, although further escalation of the C dose may be feasible since DLT was not seen at this dose level.

Dose Levels

Dose Level	# Patients/Evaluable	# DLT	Type of DLT
DL 1 :V 50mg , C 50mg	3/3	0	
DL 2 :V 100 mg, C 50mg	4/3	0	
DL 3 :V 200 mg, C 50mg	6/6	1	Headache
DL 4 :V 300 mg, C 50mg	6/5	2	Nausea (N=2)
DL 3A :V 200 mg; C 75mg	3/3	0	
DL 3B :V 200 mg, C 100mg	6/3	0	
DL 3C :V 200 mg, C 125mg	3/3	0	

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-09

Title: Pan-HER, an antibody mixture with antitumor activity against drug-resistant HER2-overexpressing breast cancers with high ERBB ligand expression

Schwarz LJ J, Hutchinson KE E, Estrada MV V, Sanders ME E, Dugger TC C, Formisano L, Guerrero AL L, Red-Brewer M, Young CD D, Lantto J, Pedersen MW W, Kragh M, Horak ID D and Arteaga CL L. Vanderbilt University Medical Center, Nashville, TN and Symphogen, Ballerup, Denmark.

Body: Background: Amplification/overexpression of ERBB receptors and/or ligands has been associated with resistance to anti-HER2 therapies. Pan-HER is a mixture of six antibodies targeting each of the ERBB receptors, EGFR, HER2 and HER3, with synergistic pairs of antibodies. Each pair of antibodies simultaneously blocks ligand binding and/or induces target degradation, thus preventing compensatory mechanisms to anti-ERBB therapies. We examined the antitumor activity of Pan-HER against drug-sensitive and -resistant HER2+ breast cancer cells and xenografts.

Results: Pan-HER exhibited potent growth inhibitory activity against a panel of HER2+ breast cancer cells (BT474, MDA-453, MDA-361, SUM190, HCC1954, UACC893 and SKBR3). Growth inhibition was associated with internalization and degradation of EGFR, HER2 and HER3. Pan-HER was superior to the combination of trastuzumab/pertuzumab (TP) against HER2+/PIK3CA mutant MDA-361, HCC1954, UACC893 and MDA-453 cells. We next compared the effect of Pan-HER against BT474, HCC1954 and MDA-361 xenografts established in nude mice to that of trastuzumab/lapatinib (TL), TP and T-DM1. All treatments were effective across the panel of xenografts. In mice with MDA-361 tumors, Pan-HER and TP were superior to TL. Immunoblot analysis showed significant downregulation of EGFR, HER2 and HER3 only in tumors treated with Pan-HER. After a complete response, treatment was discontinued. Among mice with BT474 xenografts treated with TP, TL and T-DM1, 25-50% of mice exhibited a tumor recurrence within 50 weeks of follow-up, while no recurrences were registered in mice treated with Pan-HER. Tumors recurring after TP and T-DM1 expressed significantly higher HER3 and P-HER3 protein levels and NRG1 mRNA levels. HCC1954 xenografts recurring after T-DM1 also overexpressed NRG1 mRNA compared to tumors before therapy. We next examined the effect of Pan-HER against trastuzumab-resistant HR6 (BT474) cells (Ritter *et al.* *CCR* 2007) and HCC1954 and UACC893 cells with acquired resistance to T-DM1 (TDR; IC₅₀ >5-, >6- and 600-fold in HR6, UACC893-TDR and HCC1954-TDR cells, respectively, vs. parental cells). All T-DM1-resistant cells expressed significantly higher HER3 and P-HER3 protein levels and NRG1 mRNA and protein levels. Treatment with the HER3 neutralizing antibody LJM716 resensitized HR6 and HCC1954-TDR cells to T-DM1, suggesting a causal association between the NRG1-HER3 axis and drug resistance. Mice with HR6 tumors were treated with Pan-HER, TL, TP and T-DM1. Only Pan-HER arrested HR6 tumor growth and downregulated EGFR, HER2, HER3, P-HER3 and P-AKT. Finally, HCC1954-TDR tumors rapidly grew *in vivo* despite treatment with T-DM1. Administration of Pan-HER to mice bearing HCC1954-TDR xenografts growing in the presence of T-DM1, induced rapid tumor regressions.

Conclusions: These data suggest that multitarget therapeutic interventions, such as Pan-HER, which simultaneously remove and/or block all ERBB receptors and ligands, are a feasible and effective approach against HER2-overexpressing cancers both sensitive and resistant to anti-HER2 therapies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-10

Title: Bone seeking matrix metalloproteinase-2 inhibitors prevent bone metastatic breast cancer growth

Tauro M, Laghezza A, Tortorella P and Lynch CC C. H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL and University of Bari "A. Moro", Bari, Italy.

Body: Bone metastasis is a common event during breast cancer progression. The resultant lesions are painful and currently, despite medical advances, are incurable. The progression of bone metastatic breast cancer is critically dependent on interactions with the surrounding microenvironment. Therefore, identifying the underpinning molecular mechanisms is vital for the development of new therapies.

Rationale: Gene expression analysis and validation in human and murine specimens of bone metastases revealed that matrix metalloproteinases, such as MMP-2, are highly expressed in the bone metastatic microenvironment and significantly associated with aggressive breast cancer and poorer overall survival. In bone, tumor or host derived MMP-2 contributes to breast cancer growth and does so by processing substrates including type I collagen and transforming growth factor beta (TGF β) latency proteins. These data provide strong rationale for the application of MMP-2 inhibitors to treat the disease. However, in vivo, MMP-2 is systemically expressed. Therefore, to overcome potential toxicities noted with previous broad-spectrum MMP inhibitors (MMPi), we used highly selective bisphosphonic based MMP-2 inhibitors (BMMPi) that allowed for specific bone targeting.

Methods: We utilized a novel chemical approach to synthesize bone seeking MMP inhibitors (BMMPi) on a bisphosphonic backbone, with specificity for MMP-2 in the nanomolar range (IC₅₀=140 nM).

Results: In vitro, we tested the effect of BMMPi at varying doses (1nM-100 μ M) on the viability of the major cellular components of the cancer-bone microenvironment, namely breast cancer cells, (PyMT, 4T1, MDA-MB-231, MCF-7), osteoblasts (MC3T3) and osteoclasts (primary monocytes and RAW 264.7). In vivo, we demonstrated using two bone metastatic models (PyMT-R221A-Luc and 4T1-Luc) that BMMPi treatment significantly reduced tumor growth and tumor associated bone destruction. Additionally, BMMPi are superior in promoting tumor apoptosis compared to the standard of care bisphosphonate, zoledronate. MMP activity was also lower in the BMMPi treated groups (using tumor burden to normalize values). μ CT/Xray/Histomorphometry analysis also illustrated the significant beneficial effects of the BMMPi in reducing the size of osteolytic lesions (up to 80% by μ CT; p<0.05). We demonstrated MMP-2 selective inhibition in the bone microenvironment using specific and broad spectrum MMP probes. Further, compared to zoledronate, BMMPi treated mice had significantly lower levels of TGF β signaling and MMP generated type I collagen carboxy-terminal (ICTP) fragments. Taken together, our data show the feasibility of selective inhibition of MMPs in the bone metastatic breast cancer microenvironment.

Conclusions. MMP-2 specific inhibition was achieved in the bone microenvironment. BMMPi significantly inhibit breast cancer growth in bone, they are able to induce breast cancer cell apoptosis and prevent cancer induced bone destruction. Given that bisphosphonates are well tolerated in the clinical setting, we predict that BMMPi could be translated to the clinical setting for the treatment and eradication of bone metastatic breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-11

Title: A randomized, double-blind, placebo-controlled window of opportunity trial evaluating clinical effects of high dose vitamin D in patients with breast cancer

Arnaut A, Robertson S, Addison C, Chang N, Pond G and Clemons M. Ottawa Hospital Cancer Center, Ottawa, ON, Canada; Ottawa Hospital Research Institute, Ottawa, ON, Canada and Ontario Clinical Oncology Group Research Centre at Juravinski Hospital, Hamilton, ON, Canada.

Body: Background: Considerable epidemiologic and preclinical laboratory data suggest that there is a role for vitamin D in breast cancer therapy through its tumor suppressive effects. Window of opportunity trials in breast cancer are a feasible way of assessing the biologic efficacy of therapies in the pre-surgical setting. It takes advantage of the current wait times (2-6 weeks) for breast cancer surgery as a “window of opportunity” to rapidly assess biological changes *in vivo* with short term administration of novel potentially therapeutic agents. The objective of this study was to assess the biologic effects of short term high dose vitamin D intake on breast tumor biology as demonstrated by changes in biomarkers of proliferation and apoptosis.

Methods: This is a prospective, randomized, double blind, placebo-controlled phase 2 trial assessing the effect of high dose (40,000 IU) of oral vitamin D3 on breast cancer biology in patients awaiting surgical management of their primary breast cancer. Eligible patients took the study drug for at least 2 weeks leading up to the day of surgery. Pre- and post- 25-OH vitamin D blood levels were obtained. In addition, tumor biomarkers including the Ki67 index (marker of proliferation) and caspase 3 (marker of apoptosis) analyzed on the original diagnostic core biopsy sample and then compared to a repeated analysis on the tissue obtained at the time of the definitive surgical procedure.

Results: 80 patients completed the study; 38 in the control group and 42 in the vitamin D group. The mean duration on the study was 19 days. Within the study cohort, 16/80 (64%) were ER positive, 55/80 (55%) were PR positive, 65/80 (61%) were Her2 negative. Mean overall baseline blood 25-OH Vitamin D levels in the study cohort was 76.4 nmol/L, which increased to 241.9 nmol/L in the vitamin D treated group ($p=0.0001$). Mean Ki67 level at baseline was 35.4% overall and there was no statistically significant difference in the ki67 obtained from the surgical specimen between the treatment group (mean = 39.3%) and the control group (41.0%). Baseline caspase 3 level was 31.2% overall and there was no statistically significant difference in the caspase 3 obtained from the surgical specimen between the treatment group (mean = 13.1%) and the control group (15.6%). However, the overall caspase 3 level (14%) obtained from the surgical specimen from both study groups was significantly lower than that at obtained from the core biopsy at baseline (31.2%) ($p=0.04$).

Conclusion: This is the first prospective randomized trial evaluating the effect of short term, high dose vitamin D on the *in vivo* markers of proliferation and apoptosis. No significant difference was seen in these markers as a result of vitamin D intake, despite significantly higher circulating levels of 25-OH vitamin D in the blood. A significant reduction in caspase 3 was noted in both study arms when comparing the biopsy sample to the surgical specimen, which could be due to a reduction in apoptosis activity or technical factors affecting the measurement of caspase 3.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-12

Title: Phase I study of the JAK 1/2 inhibitor ruxolitinib with weekly paclitaxel for the treatment of HER2 negative metastatic breast cancer (MBC)

Overmoyer B, Regan M, Schlosnagle E, Bunnell C, Freedman R, Tolaney S, Chen W, Mayer E, Partridge A, Silver D and Winer E. Dana Farber Cancer Institute, Boston, MA.

Body: Introduction: Inflammatory breast cancer (IBC) contains a predominance of CD44+/CD24- cells which are reported to be associated with disease proliferation and metastasis. The IL6/JAK2/STAT3 pathway is active and necessary for the survival of CD44+/CD24- cells. Pre-clinical data have shown that >80% of all IBC overexpress activated STAT3 (pSTAT3) and >95% of triple negative (TN) IBC specimens overexpress pSTAT3 (SABCS 2012 P4-06-01). Ruxolitinib is an approved JAK1/JAK2 inhibitor and has been studied in combination with paclitaxel in IBC PDX models resulting in a synergistic reduction in tumor weight. In anticipation of initiating a phase II study of ruxolitinib and paclitaxel for the treatment of TN IBC, conducted by the Translational Breast Cancer Research Consortium (TBCRC), we completed a phase I study of the combination in patients (pts) with MBC treated at Dana Farber/Harvard Cancer Center.

Methods: Pts with HER2 negative MBC, having acceptable hepatic, renal and hematologic status, were eligible if they had received ≤ 3 chemotherapy regimens for advanced disease. Objectives included: determination of maximum tolerated dose (MTD) and recommended phase II dose (RP2D), dose-limiting toxicity (DLT), adverse events (AE), clinical activity and association of IL6 and CRP levels with response. Pts were treated with escalating doses of ruxolitinib (10mg bid-25mg bid) in a 3+3 dose escalation design. Weekly (wk) paclitaxel (80 mg/m²/wk) was administered concurrently with ruxolitinib on a 3-wk cycle. DLT was assessed during the first 2 cycles. Pts with stable disease (SD) or disease response could discontinue paclitaxel after 4 cycles and continue on single-agent ruxolitinib until disease progression (PD). IL6 and CRP values were obtained initially and at the time of PD.

Results: 19 pts were enrolled, with a mean age of 45 years. BC was TN in 8 (42%), 11 (58%) were hormone receptor positive; 12 (63%) had visceral disease. 10 (53%) were enrolled as 1st-line therapy. Pts received a median number of 5 (range 1-12) cycles of combined therapy. 5 pts continued to receive ruxolitinib alone for 1,1,3,4 and 29 cycles. One pt with TN IBC remained on single agent ruxolitinib for 40 cycles. The MTD of ruxolitinib was 25 mg bid. 1 DLT of grade (gd) 3 osteonecrosis of the jaw occurred at 20mg bid, due to bisphosphonate use. 13 (68%) pts required dose reductions or doses held, with toxicities of neutropenia (50%), anemia (33%), fatigue (17%), edema (10%) and dyspnea (1%). 5 of the 6 pts in the 20 mg bid cohort required a dose reduction of paclitaxel prior to completing 4 cycles. The RP2D was determined to be 15 mg bid. 4 (21%) pts had partial response (PR), 12 (63%) pts had SD and 3 (16%) had PD as best response. Pts with PR received a median of 11 cycles and pts with SD received a median of 4 cycles of combination therapy.

Conclusions: The combination of ruxolitinib and weekly paclitaxel was well tolerated with evidence of clinical activity. The R2PD of 15 mg bid ruxolitinib will be used with paclitaxel 80 mg/m² wk dosing in the upcoming phase II preoperative study for TN IBC conducted by the TBCRC. Clinical trial information: [NCT02041429](https://clinicaltrials.gov/ct2/show/study/NCT02041429).

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-13

Title: Single domain antibody (SBT-100) inhibits growth of human HER2+ and triple negative breast cancers (TNBC) in xenografts by binding STAT3 and P-STAT3

Singh S, Murillo G, Chen D, Singh A, Singh S, Singh A, Mehta R and Parihar A. Singh Biotechnology, Tampa, FL; IIT Research Institute, Chicago, IL and Creative Biolabs, Shirley, NY.

Body: SBT-100 is a single domain antibody (sdAb), developed by Singh Biotechnology, that binds unphosphorylated signal transducer and activator of transcription 3 (STAT3) and phosphorylated STAT3 (P-STAT3). SBT-100 is approximately 13 kD or less than 1/10th the size of a human IgG molecule, and is able to cross the cell membrane to bind intracellular STAT3 and P-STAT3. This in turn inhibits its effects on genes that promote malignant behavior of cancer cells. SBT-100 has a short serum half-life but a long biological half-life. Since certain types of human breast cancers express P-STAT3, we wanted to determine if SBT-100 could inhibit the growth of human breast cancers in vitro and in vivo by studying its effects on MCF-7 (ER+/PR+), BT474 (HER2+), and MDA-MB-231 (TNBC) cells.

BACKGROUND: Many different types of human cancers (solid tumors, leukemias, and lymphomas) are dependent on constitutive expression of (P-STAT3) for their malignant phenotype. Growth factors, tyrosine kinase receptors, cytokines (IL-6, IL-11, IL-12, IL-23), BCR-ABL, and Src are some ways that STAT3 can be activated. In turn P-STAT3 turns on genes such as Cyclin D1 & D3, MMPs, Bcl-xL, Mcl-1, survivin, VEGF, and HIF-1 alpha. Constitutive expression of P-STAT3 has been shown to promote cancer cell proliferation, survival, angiogenesis, immune suppression, and metastasis. Additionally there is increasing evidence suggesting that unphosphorylated STAT3 contributes to malignant phenotype of cancers. STAT3 is also important for the survival of cancer stem cells as well as for some human breast cancers.

METHODS: Immunoprecipitation and Western blot analyses were carried out to test whether SBT-100 binds cytoplasmic STAT3 and P-STAT3 in various malignant cell lines (e.g., MDA-MB-231, PANC-1, DU145, and HeLa). MTT assays were done to determine if SBT-100 could suppress the growth of different types of human breast cancers in vitro. Xenograft cancer models using ER+/PR+ (MCF-7), HER2+ (BT474), and TNBC (MDA-MB-231) cancer cells were used to evaluate treatment with SBT-100 1mg/kg/BID (IV and/or IP route).

RESULTS: Immunoprecipitation and Western blot studies demonstrated that SBT-100 binds to both STAT3 and P-STAT3 in human cancers cells (MDA-MB-231, PANC-1, DU145, and HeLa). In a three day MTT assay, at least 90% growth suppression was achieved for all three subtypes of human breast cancer, which is highly significant. In the xenograft cancer models, SBT-100 (1mg/kg/BID) treatment for 28 days, yield growth suppression as follows: MDA-MB-231 44.8% (p<0.05) versus its control group and BT474 52% (p<0.07). While the MCF-7 xenograft cancer model showed no suppression.

CONCLUSION: Singh Biotechnology's novel sdAb, SBT-100 suppresses growth of TNBC and HER2+ human breast cancers in vivo and suppresses growth of ER+/PR+, HER2+, and TNBC cells in vitro. The most significant anti-cancer effects of SBT-100 is observed against human TNBC.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-14

Title: Proteasome inhibitors prevent bi-directional HER2/estrogen-receptor cross-talk leading to cell death in endocrine and lapatinib-resistant HER2+/ER+ breast cancer cells

Thaler S, Schmidt M, Thiede G, Schad A and Sleeman JP P. Centre for Biomedicine and Medical Technology Mannheim (CBTM), Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany; University Medical Center, Johannes Gutenberg University, Mainz, Germany; Institute of Pathology, University Medical Center, Johannes Gutenberg University, Mainz, Germany and Karlsruhe Institute of Technology Campus Nord, Institute for Toxicology and Genetics, Karlsruhe, Germany.

Body: Background: Aberrant signaling through HER2 and other members of the HER family has been identified as mediator of endocrine resistance in estrogen receptor alpha (ER α) positive breast cancer. On the other hand, ER α co-expression has been shown to attenuate the efficiency of anti-HER2 targeted therapies. These findings indicate that HER2 and ER α synergize to allow breast cancer cells to escape from both anti-ER α and anti-HER2-targeted therapies. Rationally designed clinical trials that combine endocrine therapy with anti-HER2 agents to interfere with HER2/ER α cross-talk have been conducted. However, the outcome of these trials suggests that novel therapeutic approaches are needed to further improve inhibition of HER2 and other HER family members in conjunction with a more efficient ER α blockade. We examined the ability of proteasome inhibitors (PIs) to disrupt HER2/ER α cross-talk in HER2+/ER+ breast cancer (BC) cells. Furthermore we investigated the potential of PIs to suppress the activity of a constitutively active HER2 variant resistant to trastuzumab and lapatinib.

Methods: HER2+/ER+ BC cells and fulvestrant resistant ER+ BC cells that overexpress a constitutively active HER2 variant resistant to trastuzumab and lapatinib have been treated with the PIs carfilzomib and bortezomib. The potential of these PIs to suppress ER α expression, to block HER2 activation and to inhibit the HER2 downstream pathways PI3K/Akt and Ras/MAPK was monitored by western blotting. Induction of cell death upon PI treatment was measured by quantification of SubG1 cells using propidium iodide staining or the use of colony formation assays.

Results: Carfilzomib and bortezomib markedly inhibit bi-directional HER2/ER α signaling pathways in HER2/ER+ BC cells. Both PIs suppress ER α expression, inhibit HER2 activity and subsequently suppress the HER2 downstream pathways PI3K/Akt and Ras/MAPK that are major executors for endocrine resistance. Furthermore we observed that both PIs stabilize the HER2 specific tyrosine phosphatase BDP1 (PTPN18), thereby suppressing the activity of even a constitutive active HER2 variant that cause resistance to trastuzumab and lapatinib. Based on these findings we hypothesize that PIs inhibits ER α and HER2 activity through different mechanisms as currently used therapeutic regimens.

Conclusion: These findings demonstrate that PIs disrupt the cross-talk between HER2 and ER α signaling pathways and therefore might have the potential to expand treatment opportunities for HER2+/ER+ and possibly also for other groups of BC patients.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-15

Title: Efficacy results of a phase 1/2 study of glucocorticoid receptor (GR) antagonist mifepristone (MIFE) in combination with eribulin in GR-positive triple-negative breast cancer (TNBC)

Han HS S, Wilks S, Paplomata E, Modiano MR R, Becerra C, Braiteh FS S, Spira AI I, Pluard TJ J, Richards DA A, Conzen SD D, Baker G, Fishman RS S, Marcantonio A, O'Shaughnessy J and Nanda R. Texas Oncology San Antonio; ACRC/Arizona Clinical Research Center and Arizona Oncology; Virginia Cancer Specialists Research Institute; Texas Oncology - Baylor Charles A Sammons Cancer Center; Moffitt Cancer Center; The US Oncology Network/Mckesson Specialty Health; Emory University Winship Cancer Institute Midtown; Saint Luke's Cancer Institute; Tyler Cancer Center, US Oncology Research; Baylor University Medical Center Texas Oncology US Oncology, Dallas, TX; Corcept Therapeutics, Inc; University of Chicago Medical Center and Beth Israel Deaconess Medical Center.

Body: Background: GR is variably expressed in TNBC and high expression is associated with poor prognosis in estrogen receptor-negative (ER-) early stage breast cancer. Treatment with mifepristone (MIFE) potentiates the effectiveness of chemotherapy in GR+ TNBC xenografts. Enrollment is complete in this study of patients with GR+ TNBC treated at the recommended Phase 2 dose (RP2D) of MIFE in combination with eribulin. **Objectives:** To determine the safety, tolerability, pharmacokinetics (PK) and clinical activity of the MIFE plus eribulin combination in pts with GR+ TNBC at the RP2D. **Methods:** Eligibility: In Part 1 (dose finding), pts with solid tumors; in Part 2 (expansion phase), pts with TNBC (GR result required at time of screening in Part 1, but could be pending at time of screening in Part 2). Up to 5 prior chemotherapy regimens for advanced disease; ECOG PS 0-1; adequate end-organ function. Design: 3 + 3 dose escalation scheme. After a 7-day lead-in of oral daily MIFE alone, MIFE was continued daily and eribulin was given on days 1 and 8 of a 21-day cycle. GR+ was defined as >10% of tumor cells with any intensity of GR staining. **Results:** 16 pts with metastatic breast cancer were treated in Part 1, and 21 pts with TNBC were treated in Part 2. Median age was 54 (range 30-81). MTD/RP2D was MIFE 300 mg/day + eribulin 1.1 mg/m². Safety: DLT in Part 1 was neutropenia. Neutropenia occurred in 23/36 total patients (2 Grade [G] 1, 10 G3, 11 G4); 2 instances included neutropenic fever. Recovery of WBC was brisk with growth factor support. Neuropathy was observed in 8 pts (5 G1, 1 G2, 2 G3). Other most common AEs (fatigue, hypokalemia, nausea, alopecia) were mainly G1 or G2; among these, G3/G4 events were limited to fatigue (4 G3), hypokalemia (3 G3 and 1 G4) and nausea (1 G3). There were 2 instances of G1 vaginal bleeding. There was no impact of MIFE on eribulin PK. Efficacy: There were 23 evaluable pts with TNBC across Parts 1 and 2 treated at the RP2D: 21 GR+, 2 GR status unknown; median of 3 prior chemotherapy regimens; 1 patient had received prior eribulin. Responses were: 3 PR, 8 SD, 11 PD and one too early to assess. Median PFS was 9 weeks. **Conclusions:** MIFE plus eribulin was well tolerated and appears to be an active treatment regimen. Five TNBC patients had a PFS longer than the upper 95% CI for PFS (i.e., >15 wks) reported by Aogi et al. for TNBC treated with eribulin (Annals of Oncology 2012;23:1441-48). Clinical trial information: NCT02014337.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-16

Title: Delivery and anti-tumor activity of nanoliposomal irinotecan (Nal-IRI, MM-398) in metastatic xenograft models of triple negative breast cancer

Lee H, Ventura M, Bernards N, Mohammad AS S, Foltz W, Fitzgerald J, Jaffray DA A, Lockman PR R, Zheng J and Hendriks BS S. Merrimack Pharmaceuticals, Inc., Cambridge, MA; TECHNA Institute for the Advancement of Technology for Health, Toronto, ON, Canada; West Virginia University HSC, School of Pharmacy, Morgantown, WV; University of Toronto, Toronto, ON, Canada and Institute of Biomaterial and Biomedical Engineering, University of Toronto, Toronto, ON, Canada.

Body: Introduction: Nal-IRI (MM-398, nanoliposomal irinotecan), is designed for extended circulation relative to nonliposomal irinotecan and to exploit leaky tumor vasculature for enhanced drug delivery to tumors. Nal-IRI (ONIVYDE®), in combination with 5-fluorouracil and leucovorin, was recently approved for the treatment of patients with advanced pancreatic cancer who have been previously treated with gemcitabine. A pilot clinical study established ferumoxytol (FMX) measured by magnetic resonance imaging (MRI) as a potential surrogate for assessing nanoparticle delivery to tumors. Lesions with FMX uptake above the median were associated with greater reductions in tumor size following treatment with nal-IRI. Nal-IRI with FMX MRI is currently being evaluated in patients with metastatic breast cancer (NCT01770353). Here we present preclinical data demonstrating the activity of nal-IRI monotherapy in multiple models of triple negative breast cancer (TNBC).

Methods: Imaging and treatment efficacy studies were carried out in the MDA-MB-231 derived mouse models of TNBC, including the LM2-4 variant with spontaneous metastases. Delivery of nal-IRI to brain metastases was assessed in MDA-MB-231-Br-Luc model (intracardiac implantation) using fluorescently labeled nal-IRI. Kinetics of FMX tumor uptake were evaluated with 7T MRI. Total tumor irinotecan and the active metabolite SN-38 were quantified by high performance liquid chromatography.

Results: Nal-IRI treatment was well-tolerated based on body weight monitoring. In the LM2-4 model, nal-IRI (10 mg/kg salt) was more effective in suppressing primary tumor regrowth (median tumor volume of 155 mm³ vs. 946 mm³ at day 14), reducing metastatic burden (median bioluminescence flux of 0.4x10⁹ vs. 2.1x10⁹ at day 12), and prolonging overall survival (median survival of 66 days vs. 14 days), compared to nonliposomal irinotecan (50 mg/kg salt). Similarly, nal-IRI demonstrated benefits in reducing brain metastatic burden and extended survival compared to untreated control in the MDA-MB-231 brain metastasis model. Fluorescence microscopy revealed that nal-IRI primarily localized in the metastatic lesions, with undetectable signal in normal brain tissue. At 24 h post FMX-injection, FMX uptake correlated positively with tumor SN-38 levels at 24 h following treatment with nal-IRI (p = 0.0222, Spearman correlation), supporting that nanoparticle imaging may be useful as a surrogate measure of nal-IRI tumor delivery. Furthermore, higher tumor FMX deposition was associated with increased tumor growth inhibition with nal-IRI, corroborating observations from the pilot Phase 1 clinical study.

Conclusions: These studies support the evaluation of nal-IRI in patients with TNBC, as well as further evaluation of nanoparticle imaging as a potential biomarker for identifying lesions likely to respond.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-12-17

Title: H-ferritin allows nanometronomic treatment of breast cancer with doxorubicin preventing drug resistance and circumventing cardiotoxicity

Mazzucchelli S, Fiandra L, Bellini M, Truffi M, Rizzuto MA A, Sorrentino L, Longhi E, Nebuloni M, Prospero D and Corsi F. University of Milan, Department of Biomedical and Clinical Sciences "L. Sacco", Milan, Italy; University of Milan-Bicocca, Milan, Italy and IRCCS S. Maugeri Foundation, Pavia, Milan, Italy.

Body: Chemotherapeutic treatment of breast cancer is based on maximum tolerated dose (MTD) approach.¹ However, advanced stage tumors are not effectively eradicated by MTD owing to suboptimal drug targeting, onset of therapeutic resistance and neoangiogenesis. In contrast, "metronomic" chemotherapy is based on frequent drug administrations at lower doses, resulting in neovascularization inhibition and induction of tumor dormancy.^{1,2} However, several limiting factors remain for LDM in order to displace MTD treatments in clinical practice, including 1) low drug accumulation at tumor site,² 2) controversial effectiveness against chemoresistance in advanced metastatic cancers, and 3) acquired resistance after prolonged treatment. Recent advances in nanotechnology could offer groundbreaking solutions to improve the effectiveness of LDM chemotherapy, by taking advantage of the unique targeting efficiency of engineered nanocarriers.³

Here, we propose a new concept of "nanometronomic" chemotherapy, exploiting the H-ferritin (HFn)-mediated targeted delivery of doxorubicin (DOX) in an aggressive and metastatic breast cancer mouse model with DOX-inducible chemoresistance. HFn nanocages naturally target cancer cells⁴ owing to its affinity for transferrin receptor 1. HFn-DOX was recently demonstrated to overcome chemoresistance by actively promoting DOX nuclear translocation *in vitro*^{5,6} and was tested as a MTD treatment on a DOX-sensitive tumor model with encouraging results.⁷ We find that LDM administration of HFn-DOX strongly improves the antitumor potential of DOX chemotherapy arresting the tumor progression. Indeed, *in vitro* and *in vivo* results demonstrate that HFn nanocages mediate the nuclear delivery of DOX and increase DOX accumulation both in tumor tissue and in cancer cell nuclei, resulting in increased efficacy. Moreover, we find that HFn-DOX antitumor effect is attributable to multiple nanodrug actions beyond cell killing, including inhibition of tumor angiogenesis and avoidance of chemoresistance. Otherwise, although an even better reduction of tumor progression was achieved with liposomal DOX (pl-DOX) a five-fold increase in MDR-1-positive cells has been displayed, suggesting that liposomal DOX is not suitable in view of a protracted metronomic treatment, due to the onset of chemoresistance. Multiparametric assessment of heart tissues, including histology, ultrastructural analysis of tissue morphology, and measurement of markers of reactive oxygen species and hepatic/renal conditions, provided evidence that metronomic HFn-DOX allowed us to overcome cardiotoxicity contrary to what is observed with DOX and pl-DOX. Our results suggest that HFn-DOX has tremendous potential for the development of "nanometronomic" chemotherapy toward safe and tailored oncological treatments.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P6-13-01

Title: Proteomic response in breast cancer treated with neoadjuvant chemotherapy with and without bevacizumab: Reverse phase protein array (RPPA) results from NeoAva - A randomized phase II study

Haugen MH H, Lindgjærde OC, Krohn M, Zhao W, Lindholm EM M, Silwal-Pandit L, Borgen E, Garred Ø, Fangberget A, Holmen MM M, Schlichting E, Skjerven H, Lundgren S, Wist E, Naume B, Mælandsmo GM M, Lu Y, Børresen-Dale A-L, Mills GB B and Engebråten O. Oslo University Hospital, Oslo, Norway; University of Oslo, Oslo, Norway; MD Anderson Cancer Center, Houston, TX; Vestre Viken Hospital Trust, Drammen, Norway and St. Olavs Hospital, Trondheim, Norway.

Body: BACKGROUND: Patients with HER2 negative primary tumors of ≥ 25 mm were treated with neoadjuvant chemotherapy (4 x FEC100 + 12 weeks of taxane-based therapy) and randomized (1:1) to receive bevacizumab or not. Mammography, ultrasound and MR imaging were used for response evaluation, in addition to the final pathology assessment after surgery.

HYPOTHESIS: RPPA proteomic analyses support identification of molecular mechanisms associated with clinical response to bevacizumab treatment.

METHODS: Tumor responses were evaluable in 132 patients; of which 66 received bevacizumab. Ratio of the tumor size at final pathology assessment, and at inclusion was calculated to obtain a continuous scale of response reflecting the percentage of tumor shrinkage in response to therapy. Tumor material was obtained at screening, 12 weeks into treatment and at surgical removal of tumors at 25 weeks. Lysates from each sample was analyzed on reverse phase protein arrays (RPPA) for expression levels of 210 proteins of which 54 were phospho-specific.

RESULTS: Several proteins were found for which expression prior to treatment reflected a better response on tumor shrinkage in the combination treatment arm (chemotherapy+bevacizumab). The proteomic response from week 0 to 12 in both treatment arms had an overall similar profile regarding up- and down-regulated proteins; however, the combination treatment (FEC100 + bevacizumab) induced a more pronounced effect on regulation of each protein. This might reflect the capability of bevacizumab therapy to potentiate the effects of the anthracyclin based chemotherapy from week 0 to 12. Conversely, from week 12-25 (taxane-based therapy + bevacizumab) this effect was lost or even reversed, except for certain phosphoproteins where potentiation imposed by bevacizumab was sustained throughout the whole treatment period. We are in the process of analyzing the impact of phosphorylation and thus protein activation states on treatment response. Furthermore, tumors with low hormone receptor pathway score demonstrated a better response in the combination treatment (chemotherapy+bevacizumab).

Additionally, in these good responders the hormone signaling pathway was significantly upregulated during treatment. Further investigations are conducted to determine if this was due to selective ablation of hormone receptor negative tumor cells, or a re-programming of the molecular phenotype of cells present prior to treatment. The above mentioned results have potentially important clinical relevance and will be further investigated with respect to subtypes and the biological pathways affected by antiangiogenic therapy.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-13-02

Title: Efficacy of first-line bevacizumab (BEV)-containing therapy for poor-prognosis advanced breast cancer (aBC): Subgroup analyses of the German AVANTI observational study

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Body: Background: The multicenter AVANTI observational study is evaluating the safety and effectiveness of EU-approved BEV-containing regimens (BEV + paclitaxel [PAC] or BEV + capecitabine [CAP]) as first-line therapy for HER2-negative aBC in German routine oncology practice.

Methods: Eligible patients (pts) had received no prior chemotherapy (CT) for aBC and had no BEV contraindications. CT schedule, diagnostics, and frequency of follow-up visits are at the physician's discretion. Data are collected for 1 year after starting BEV, with 6-monthly follow-up for 1.5 years thereafter. We explored treatment outcomes in pts with triple-negative aBC (TNBC), pts considered at high risk according to a simple prognostic index for OS in BEV-treated pts [Llombart, 2014], and subgroups defined by timing of BEV+CT initiation.

Results: Between Oct 2009 and Feb 2015, 2168 pts treated at 331 centers received BEV+PAC (n=1774) or BEV+CAP (n=394). Of these, 445 (21%) had TNBC and 306 (14%) met the high-risk criteria. Within the hormone receptor-positive (HR+) subgroup, pts receiving endocrine therapy (ET) before BEV+CT were older than pts starting BEV+CT immediately (median age 65 vs 60 years, respectively) and included a smaller proportion with ECOG performance status 0 (39% vs 47%), visceral metastases (70% vs 77%), or prior (neo)adjuvant CT exposure (46% vs 57%). In all subgroups, median BEV treatment duration was longer than median CT duration. At the data cutoff for this interim analysis (Mar 1, 2015), median duration of observation was 10.8 (range <0.1–47.5) months. The table shows treatment exposure and efficacy overall and in selected subgroups.

table 1

	All pts (n=2168)	TNBC (n=445) ^a	HR+ with immediate BEV+CT (n=1260) ^{a,b}	HR+ with ET before BEV+CT (n=309) ^{a,b}	High risk ^c (n=306) ^b
BEV+PAC, n (%)	1774 (82)	352 (79) ^b	1062 (84)	238 (77)	229 (75)
Treated until PD, n (%) ^{b,d}	640 (30)	180 (40)	314 (25)	104 (34)	125 (41)
BEV+CT until PD	449 (21)	143 (32)	210 (17)	61 (20)	99 (32)
Single-agent BEV until PD	191 (9)	37 (8)	104 (8)	43 (14)	26 (8)
Median BEV duration, months (95% CI)	5.9 (5.6–6.3)	5.1 (4.9–5.6)	6.4 (5.9–7.0)	5.6 (5.1–6.5)	5.1 (4.6–5.6)
Median CT duration, months	4.6 (4.4–4.9)	3.9 (3.5–4.2)	4.9 (4.6–5.1)	4.6 (4.2–5.1)	3.9 (3.3–4.4)
No. of PFS events/pts (%) ^e	1238/2154 (57)	302/441 (68)	667/1255 (53)	187/307 (61)	210/306 (69)
Median PFS, months (95% CI)	10.1 (9.7–10.7)	7.2 (6.2–8.0)	11.5 (10.8–12.3)	9.0 (8.3–10.0)	6.4 (5.9–7.4)

^a154 pts could not be classified as TNBC or HR+ because of missing HR status information. ^bPost hoc analysis. ^c≥3 of the 5 risk factors (disease-free interval ≤24 months; ECOG performance status ≥2; liver metastases and/or ≥3 metastatic organ sites; TNBC; prior (neo)adjuvant anthracycline and/or taxane). ^dData available only in pts with documented end of treatment. ^ePFS data missing in 14 pts.

Conclusions: Interim results from this large observational study indicate that first-line BEV+CT is an effective therapy in all risk subgroups of a general population of pts with HER2-negative aBC treated in routine oncology practice, including pts with a particularly poor prognosis. Results of these exploratory subgroup analyses suggest that BEV+CT could be considered irrespective of HR status.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-14-01

Title: Enhanced efficacy of redirected T-cell therapy of TNBC with a Trop-2/CD3 bispecific antibody in combination with a checkpoint inhibitor

Rossi EA A, Chang K, Cardillo TM M, Rossi DL L, Li R, Mostafa A, Sharkey RM M and Goldenberg DM M. Immunomedics, Inc., Morris Plains, NJ.

Body: Bispecific antibodies (bsAbs) for redirecting T cells to cancers have shown promise in both pre-clinical and clinical studies. However, clinical success has been minimal for solid cancers to-date. Previously, we reported highly effective T-cell redirected therapy of pancreatic and gastric tumors in xenograft models using a trivalent bsAb, designated (E1)-3s, which comprises an anti-CD3 scFv covalently conjugated to a stabilized dimer of a Trop-2-targeting humanized Fab. Trop-2 is highly expressed in diverse epithelial cancers, including breast, lung, gastric, colorectal, pancreatic, bladder, ovarian, uterine, and prostate carcinomas, with limited presence on normal human tissues. Compared to first generation bsAbs (e.g., BiTE), which induce high-level cytokine production that can lead to serious side effects, efficient T-cell killing is mediated by (E1)-3s with minimal cytokine release. Herein, we report the potential utility of (E1)-3s for therapy of breast cancers, including TNBC. Additionally, we show that addition of a checkpoint inhibitor can enhance bsAb-redirected T-cell therapy. IMMU-cPD1 is a chimeric mAb that binds with high affinity to human PD-1 and efficiently blocks binding to its ligand, PD-L1. The MDA-MB-231 human TNBC cell line has relatively low levels of surface Trop-2 (36,000/cell) and expresses PD-L1 constitutively. In *ex-vivo* assays, where MDA-MB-231 cells were mixed with purified human T cells, (E1)-3s mediated potent T-cell redirected killing ($IC_{50} < 10 \text{ pM}$). Addition of IMMU-cPD1 enhanced (E1)-3s-mediated T-cell killing. The advantage of combining the checkpoint inhibitor with redirected T-cell therapy was supported with *in-vivo* xenograft studies. NOD-SCID mice were co-injected with purified human T cells (2.5×10^6) and MDA-MB-231 (5×10^6). Groups were administered: 1) five daily injections of (E1)-3s; 2) cPD-1 twice weekly for 4 weeks; or 3) a combination of (E1)-3s and cPD1 treatments. An untreated control group comprising T cells and tumor cells reached the endpoint (tumors $>1 \text{ cm}^3$) with a median survival time (MST) of 35 days. The group treated with (E1)-3s had significantly smaller tumors ($P=0.0023$, AUC) and improved MST (42 days, $P=0.0019$). The group treated with the combination of (E1)-3s and IMMU-cPD1 had significantly smaller tumors ($P=0.0121$, AUC) and longer MST (49 days, $P=0.008$), compared to those treated with (E1)-3s alone. Treatment with IMMU-cPD1 alone did not retard tumor growth or improve MST, compared to the untreated control group. In conclusion, (E1)-3s is an attractive candidate for T-cell redirected therapy of breast cancer due to its potent activity with potentially reduced side effects and the prevalence of Trop-2 expression associated with this disease. Tumor microarrays representing 117 breast cancer patients showed $>85\%$ positivity for Trop-2. Our immunohistochemical analysis of more than 50 individual TNBC patient specimens demonstrated 92% positivity, with 80% having moderate to strong Trop-2-staining. Combining checkpoint inhibitors with redirected T-cell therapy may represent a new paradigm for the management of solid cancers, including breast, and is worthy of further investigation.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-14-02

Title: An anti-PD1 antibody-based therapy results in dramatic reduction of TNBC PDX tumors in humanized mice models

Rosato RR R, Davila-Gonzalez D, Choi DS Soon, Dave B and Chang JC C. Houston Methodist Cancer Center, Houston, TX.

Body: Recently, the field of cancer immunotherapy has seen a rapid growth based on a better understanding of the complex interplay between the tumor and the immune system. Although for long time breast cancer has been considered non-immunogenic and patients have seen limited options to immunotherapies, new strategies have changed this paradigm. In the present study, we aimed to test the in vivo activity of a human anti-PD1 antibody against the TNBC tumor line MC1. One of the main limitations of performing laboratory-based in vivo studies resides in the availability of the appropriate animal models. To circumvent these obstacles, we used patient-derived breast cancer tumor lines xenografts (PDX) from our existing collection previously established in immuno-compromised SCID/beige mice. Low-passage fresh xenograft tumor fragments of the TNBC tumor lines MC1 and HM#2147 were transplanted into the cleared fat pad of recipient non-humanized (non-hNSG) and humanized NSG (hNSG) mice. Humanized mice were obtained by i.v. injecting 3-4 weeks old NSG mice with CD34+ hematopoietic stem cells (HSC) following whole body radiation. Flow cytometry and immuno-histochemistry analyses of hNSG blood, spleen and bone marrow showed the presence of human CD45+ (15.1% ± 10.3; 61.5% ± 19.1; 71.9% ± 17.9; respectively), CD20+, CD3+, CD8+, CD68+, and CD33+ cells. BC tumor engraftment was then evaluated by comparing the growth of the MC1 tumor line in non- and hNSG mice, showing a slower growth in the corresponding humanized mice. Importantly, the presence of hCD45+ cells was readily detectable in all the hNSG-derived tumors, localizing both toward the periphery of the tumors and inside them. Analysis of hCD45+ subpopulation cells showed also the tumor presence of hCD20+ cells (B cells), hCD8+ T-cells and CD68+ (macrophages) cells. To determine whether BC PDX may have conserved the capability to metastasize to the lung, hNSG mice were engrafted with the tumor line HM#2147. Once the primary tumor reached the maximum volume allowed by humane standards, mice humanization levels, tumor engraftment and lung metastasis were evaluated. Humanized engrafted mice showed same levels of human cells and primary tumor engraftment as those harboring MC1 PDXs. Macroscopically, lungs displayed clear evidence of metastases. IHC assays using Ki67 and CK19 identified the microscopic region corresponding to its localization. Importantly, as described in the primary breast tumor, the presence of hCD45+ was also observed infiltrating the lung metastatic tumor. The efficacy of an anti-PD1 therapy was then evaluated. Levels of tumor PD-L1 were determined by western blot showing high levels of expression. Animals were weekly i.p.-administered either the human anti-PD1 antibody or vehicle. Evaluation of tumor volumes showed a significant reduction in anti-PD1- vs. vehicle-treated animals at day 18 of treatment (i.e. 457.8 mm³ vs. 1074.24 mm³, respectively; *P*= 0.001). The present study show encouraging results associated with anti-PD1 immunotherapy to treat TNBC tumors. In addition, our results provide evidence supporting the use of humanized mice as key animal model that may allow to overcome some of the technical difficulties associated with the investigation of immune-based therapies.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-15-01

Title: Analysis of renal function in MONARCH 1: A phase 2 study of abemaciclib, a CDK4 & 6 inhibitor, as monotherapy, in patients with HR+/HER2- breast cancer, after chemotherapy for metastatic breast cancer (MBC)

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Body: Background: Abemaciclib is an oral, selective inhibitor of CDK4 & 6 that has demonstrated activity in different tumor types. In a phase 1 trial, treatment emergent adverse events (TEAEs) of increased creatinine, grade 1 or 2 severity by Common Terminology Criteria for Adverse Events (CTCAE v4.0) were reported in > 10% of patients (pts) who received abemaciclib monotherapy¹. In vitro, abemaciclib and its major metabolites inhibit renal transporters OCT2, MATE1, and MATE2-K².

Methods: MONARCH 1 is a phase 2 single-arm study designed to evaluate safety and efficacy of abemaciclib monotherapy in women with HR+/HER2- MBC whose disease progressed on/after endocrine- and chemotherapy. Abemaciclib (200 mg) was administered orally on a continuous schedule every 12 hours until disease progression or unacceptable toxicity occurred. We retrospectively analyzed changes in serum creatinine, blood urea nitrogen (BUN), cystatin C, and calculated glomerular filtration rate (GFR) based on cystatin C using central lab values. TEAEs (CTCAE v4.0), dose delays, and treatment discontinuation associated with renal events were examined.

Results: Of the 132 pts treated, 130 pts had central laboratory data available, and 128 pts (98.5%) experienced an increased serum creatinine: 61 pts (46.9%) grade 1, 66 pts (50.8%) grade 2, 1 pt (0.8%) grade 3. Creatinine increases occurred during cycle 1 and remained elevated but stable during treatment. Serum creatinine decreased following treatment discontinuation. No changes in mean BUN, cystatin C or estimated GFR were observed.

TEAEs of increased serum creatinine were reported in 17 pts (12.9%); one was grade 3. Due to increased serum creatinine, 2 pts experienced dose reductions, 2 pts dose omissions, and 1 pt treatment discontinuation. Serious AEs (SAEs) of increased creatinine were experienced by 4 patients; 3 were possibly related to abemaciclib. A non-drug related SAE of acute kidney injury was reported for 1 pt. Review of all reported AEs/SAEs failed to indicate any evidence of renal impairment.

The Standardized MedDRA Queries (SMQ) acute renal failure was used to search and identify all reported cases of increased creatinine with or without acute kidney injury. Twenty pts (15.2%) who experienced an AE were included in this category: 4 pts (3.0%) met the narrow term SMQ, and 17 pts (12.9%) the broad term SMQ.

Conclusions: Safety data from the MONARCH 1 study shows a causal association of the reversible increased blood creatinine with abemaciclib therapy, but not as a result of renal injury, renal insufficiency, or impaired renal function. The rise in serum creatinine is consistent with in vitro data indicating that abemaciclib is a competitive inhibitor of renal efflux transporters of creatinine².

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2016 San Antonio Breast Cancer Symposium

Publication Number: P6-15-02

Title: Phenotypically distinct HRG positive cancer cells impact standard of care therapies in metastatic breast cancer models

Finn GJ J, Zhang H, Blois A, Mathews SE E, Kudla AJ J, Baum JS S, Demars NA A, Cieslewicz MJ J and Czibere A. Merrimack Pharmaceuticals, Inc, Cambridge, MA.

Body: ErbB3 is a member of the human epidermal growth factor receptor (ErbB or HER) family which is comprised of four receptors (ErbB1-4). A defining feature of the ErbB network is that two members of the family, ErbB2 and ErbB3, are non-autonomous. ErbB2 lacks the capacity to interact with a growth-factor ligand, whereas the kinase activity of ErbB3 is defective. Heregulin (HRG), the ErbB3 ligand, has been identified as a potent driver of proliferation and enhanced survival. HRG expression leads to a distinct tumor cell phenotype characterized by an inability to respond to the effects of numerous Standard of Care (SOC) therapies, including chemotherapies, anti-hormonal agents and other targeted therapeutics.

In surveys of HRG expression, we have shown the presence of HRG+ cells in approximately 50% of the cases of most solid tumor types. We hypothesize that these HRG+ cells are protected from the effects of SOC therapy and continue to proliferate even in the presence of SOC, resulting in limited clinical benefit. In this model, if HRG activity is blocked, HRG+ cells become susceptible to SOC, resulting in enhanced clinical benefit. Seribantumab is a fully human anti-ErbB3 monoclonal antibody designed to block HRG activity by inhibiting the binding of HRG to ErbB3. In the presence of seribantumab, HRG+ tumor cells are predicted to be able to respond to co-administered SOC therapy.

For hormone receptor positive (HR+) breast cancer, hormone deprivation strategies have proven clinical benefit in the adjuvant and metastatic settings. Unfortunately, clinical benefit from these therapies can be short-lived in some patients. Optimal clinical management of these patients requires a comprehensive molecular understanding of the drivers of rapid clinical progression. We and others have found that HRG mRNA expression measured in tumor samples defines a subgroup of patients who derive only limited clinical benefit from SOC when compared to patients whose tumors do not express HRG. This was observed in a previously published Phase 2 clinical study with exemestane, and preclinically with multiple classes of anti-hormonal agents, including letrozole and fulvestrant -- treatments that currently represent the mainstay of treatment options for HR+, HER2 negative (HER2-) advanced breast cancer.

Here we will present data supporting the hypothesis that phenotypically distinct HRG+ cells in breast cancer models persist despite treatment with SOC and various novel classes of therapy. We will also show that the addition of the anti-ErbB3 antibody seribantumab to these other therapies promotes sustained treatment responses. Continued expansion of HRG+ cells could be the key to rapid clinical progression in breast cancer patients treated with SOC therapy. These findings support the development of seribantumab in combination with anti-hormonal agents in a planned Phase 3 clinical trial in HR+, HER2- advanced breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-15-03

Title: Dual targeting of mammary tumors and tumor-associated functional limitations through inhibition of NF- κ B

Wang R, Nakshatri P, Padua MB B, Anjanappa M, Penthala N, Crook PA A, Liu J, Zimmers T and Nakshatri H. IU School of Medicine, Indianapolis, IN; Richard L Roudebush VA Medical Center, Indianapolis, IN and College of Pharmacy, the University of Arkansas, Little Rock, AR.

Body: Breast cancer progression is associated with systemic effects such as functional limitation, sarcopenia and cachexia. These effects are manifested as muscle weakness, body pain or depletion of skeletal muscle mass. Over a quarter of 2.8 million breast cancer patients in the United States experience a precachexia to cachexia syndrome. It has been reported that cancer-induced cytokines activate NF- κ B, which promotes cancer progression, metastasis, and chemoresistance. These cytokines could potentially induce NF- κ B in skeletal muscle, impair skeletal muscle function, and cause functional limitations. Therefore, NF- κ B inhibitors could serve dual purpose of inhibiting cancer progression and reducing functional limitations. In present study, we used MMTV-PyMT transgenic mammary tumor model to test therapeutic effects of the NF- κ B inhibitor, Diaminomethylparthenolide (DMAPT), an orally bioavailable NF- κ B inhibitor. We observed deteriorating physical and functional conditions in PyMT+ mice with the progression of mammary tumor. Compared to wildtype mice, PyMT+ mice with mammary tumors showed decreased fat mass and grip strength, both are markers of functional limitations. Treatment with DMAPT (100mg/kg, 5 times/week, orally), starting at 8 week-old prior to mammary tumor occurrence, delayed mammary tumor onset and slowed tumor growth rates compared to vehicle treatment. Consequently, DMAPT-treated mice showed lower systemic effects of mammary tumors on grip strength and alterations of body compositions (i.e. body fat, lean mass). Moreover, systemic treatment with DMAPT significantly increased survival trends in PyMT+ mice. These results suggest that NF- κ B is a critical signaling relay engaged by paracrine effects of breast cancer on skeletal muscle function. Mechanistic studies *in vitro* suggested that cancer-induced cytokines specifically target microRNAs in skeletal muscle. miR486 is a muscle-enriched microRNA that controls differentiation of myoblasts. In particular, conditioned media from a number of mammary tumor cell lines, including PO1058 (poorly invasive tumor cells from PyMT-WapCre-mGFP+ mice in C57BL6 background) and PO1059 (highly invasive cells from PyMT-WapCre-mGFP+ mice) reduced the levels of miR486 in the myoblast cells C2C12. Literature demonstrated that reduced miR486 in muscle is linked to musculoskeletal defects in muscular dystrophy patients. These data are consistent with previous studies in which lower circulating miR486 occurred in plasma of breast cancer patients with metastasis compared with healthy women, and as well in skeletal muscle of mammary tumor bearing animals. Further studies are essential to establish a cancer-induced cytokine, NF- κ B activation in muscle, deregulated expression and release of miR486 from muscle and cancer-associated systemic effects circuitry. In summary, mammary tumors result in changes in body composition with decreased grip strength, which may be associated with altered NF- κ B signaling pathways, and NF- κ B inhibitor DMAPT can be used as a potential candidate for the treatment of breast cancer and associated functional limitations to improve the quality of life. Additionally, circulating miR486 may serve as a useful clinical indicator of progression and systemic effects of breast cancer.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-16-01

Title: The importance of loco-regional tumor burden and surgery on survival in patients with de novo stage IV breast cancer; post-hoc analyses of protocol MF07-01

Ozmen V, Ozbas S, Karanlik H, Muslumanoglu M, Igci A, Canturk Z, Utkan NZ Zafer, Ozaslan C, Evrensel T, Uras C, Aksaz E, Soyder A, Ugurlu UM Mustafa, Col C, Cabioglu N, Bozkurt B, Sezgin E, Dagoglu T, Uzunkoy A, Dulger M, Koksal N, Cengiz O, Gulluoglu B, Unal B, Atalay C, Yildirim E, Erdem E, Salimoglu S, Sezer A, Koyuncu A, Gurleyik G, Alagol H, Ulufi N, Berberoglu U and Soran A. Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey; Guven Hospital, Istanbul, Turkey; Istanbul University, Institute of Oncology, Istanbul, Turkey; Kocaeli University, Medical Faculty, Kocaeli, Turkey; Ankara Oncology and Training Hospital, Ankara, Turkey; Uludag University, Faculty of Medicine, Bursa, Turkey; Acibadem University, Faculty of Medicine, Istanbul, Turkey; MAMER Surgery Center, Bursa, Turkey; Adnan Menderes University, Faculty of Medicine, Aydin, Turkey; Marmara University Training and Research Hospital, Istanbul, Turkey; Abant Izzet Baysal University, Faculty of Medicine, Bolu, Turkey; Ankara Numune Training and Research Hospital, Ankara, Turkey; Izmir Institute of Technology, Izmir, Turkey; University of Pittsburgh, Magee-Womens Hospital, Pittsburgh, PA; Istanbul North Anatolian State Hospital, Istanbul, Turkey; University of Inonu, Faculty of Medicine, Malatya, Turkey; Ankara Liv Hospital, Ankara, Turkey; Pamukkale University, Faculty of Medicine, Denizli, Turkey; Tepecik Training Hospital, Izmir, Turkey; Trakya University, Faculty of Medicine, Edirne, Turkey; Mdicana Sivas Hospital, Sivas, Turkey; Haydarpasa Numune Hospital, Istanbul, Turkey; Gaziosmanpasa University Medical Faculty, Istanbul, Turkey and Okmeydani Training Hospital, Istanbul, Turkey.

Body: Background: The MF07-01 trial is a multicenter phase III randomized controlled trial of treatment naive stage IV BC patients comparing loco-regional surgery (LRS) followed by appropriate systemic therapy (ST) versus ST alone.

Aims: To evaluate the importance of loco-regional tumor burden and surgery on overall survival rate in patients with de novo stage IV breast cancer.

Methods: At initial diagnosis patients were randomized 1:1 to LRS group or ST group. The surgery was a lumpectomy (L) or mastectomy (M) and sentinel lymph node biopsy (SLNB) ± axillary lymph node dissection (ALND). After surgery all patients received systemic treatment + endocrine treatment (ET) and Trastuzumab based on pathology results. The demographic, pathologic, and clinical characteristics of the patients were recorded.

Results: 274 patients were accrued; 138 in the LRS group and 136 in the ST group. The groups were comparable regarding age, BMI, HER2 neu, tumor type and size, histologic grade, and bone and visceral metastasis (all p>0.05). In the LRS group 36 patients (26%) had L+ALND, 92 patients (67%) had M+ALND and 10 patients (7%) had M+SLNB, respectively.

The patients and tumor characteristics

Patients and Tumors Characteristics and Surgical Treatment	Surgery	Systemic Therapy	P Value
Age (mean /year±SD)	51.8 ±12.6	51.5±13.6	NS
Median follow-up (25%,75%)	41.0 (24,54)	37 (18,49)	
Tumor Size (%)			
T1	8.7 (12)		NS
T2	52.2 (72)		NS
T3	21.7 (30)		NS
T4	17.4 (24)		NS
Histologic Grade (%)			
I	4.4 (6)	9.6 (10)	NS
II	39.9 (55)	31.7 (33)	NS
III	55.8 (77)	58.9 (61)	NS

Surgical Treatment			
Lumpectomy+ ALND	26 (36)	-	-
M + SLNB	7 (10)	-	-
M + ALND	67 (92)	-	--
SLNB	17 (23)	-	-
ALND	92.8 (128)	-	-
pN+	89.1 (123)	-	-
30-day mortality	1.4 (2)	1.5 (2)	0.98

SLNB-Sentinel Lymph Node Biopsy; ALND-Axillary Lymph Node Dissection; M-Mastectomy

The axillary positivity rate was 89.1%. There were 76 (55%) deaths in the LRS group and 101 (74%) in the ST group during the median 40 (20-51) month follow-up. Overall survival (OS) was 34% higher in the LRS group compared to the ST group (HR: 0.66, 95%CI 0.49-0.88; p = 0.005).

Overall survival rate was higher in LN (+) (p=0.01), tumor size<5cm (p<0.0001), and high histologic grade (HG III, p<0.008) patients who underwent axillary surgery than ST group ; OS rate was with a marginal significant level in patients without axillary involvement (pN0) in the LRS group compared with ST group (p=0.05).

Conclusion: In this subgroup analysis, we observed that patients with high grade tumor, without skin or chest wall involvement and positive axilla who underwent surgery for primary breast tumor and axilla had better overall survival than ST in de novo stage IV breast. These results can be considered in clinical research design for stratification.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-16-02

Title: Beta-adrenergic receptor blockers (BB) and increased progression free survival (PFS) in patients with advanced triple negative breast cancer (TNBC): A retrospective analysis of the ROSE/TRIO012 study

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Body: Introduction: Recent retrospective studies suggest beta-adrenergic blocking drugs are associated with improved survival in patients with a wide range of cancers. Although limited and discordant data suggest that the use of BB may increase overall survival (OS) of patients with localized breast cancer (BC), there is no information on the effects of BB in women with advanced BC, which may be a more appropriate clinical scenario to evaluate anti-cancer effects of new treatment strategies. We analyzed the relationship between BB use and clinical outcomes in the ROSE/TRIO012 study, a double-blinded multinational registration phase III trial that randomized 1,144 patients with HER2-negative advanced BC to receive first-line docetaxel in combination with ramucirumab (RAM) or placebo.

Objective: To explore the association between BB use and BC outcomes in patients participating in the ROSE/TRIO-012 trial.

Methodology: We retrospectively compared PFS, OS, Overall Response Rate (ORR) and Clinical Benefit Rate (CBR) using the ITT population, in patients who received BB during the trial with those who did not receive them. PFS and OS were estimated using the Kaplan-Meier method. PFS and OS of both treatment groups were compared using the Log-Rank test. Cox proportional models were fitted to determine the association between BB, PFS and OS. ORR and CBR in both groups were compared using the Fisher's Exact Test.

Results: 153/1,144 (13%) patients received BB during the trial. Median PFS in patients treated with BB was 10.3 months, compared to 8.3 months in patients who did not receive them (HR 0.81; 95% CI 0.66-0.99; $p=0.0379$). In patients treated with BB only after enrolment (57/153, 37%) median PFS was 15.5 months vs. 8.3 months in patients with no BB ($p=0.0005$). In the subset of patients with TNBC, median PFS was 13 months if received BB compared to 5.2 months if no BB (HR 0.52; 95% CI 0.34-0.79; $p=0.002$). No difference in PFS was observed in patients with hormone receptor positive BC. The magnitude of PFS benefit in the RAM arm was similar as the whole ITT population (HR 0.73; 95% CI 0.57-0.94; $p=0.014$). If BB received only after enrolment HR 0.50; 95% CI 0.35-0.72; $p=0.0003$). The benefit of BB intake in PFS was independent of development of treatment-emergent hypertension (hypertension occurring within 42 days of first study drug administration; HR 0.92; 95% CI 0.73-1.15; $p=0.476$) but dependent on treatment arm (HR 0.86; 95% CI 0.74-0.99; $p=0.037$). The test for interactions between BB and treatment arm was not significant ($p=0.276$). No differences in OS, ORR or CBR were seen.

Conclusions: In this exploratory post-hoc analysis of the ROSE/TRIO012 study, BB intake was associated with a significant improvement in PFS compared to patients who did not receive them, particularly in patients with TNBC and patients not previously exposed to these drugs. Further evaluation of the BB intake on BC outcomes warrants evaluation in a prospectively-designed clinical trial.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-16-03

Title: Phase 2 trial of everolimus and/or trastuzumab in hormone refractory, hormone receptor (HR)-positive, HER2-normal metastatic breast cancer (MBC)

Paplomata E, Gogineni K, Meisel J, Santa-Maria C, Yuan L, Kramer J, Bill Li X, Zelnak A, Pakkala S, Kaklamani V and O'Regan R. Winship Cancer Institute of Emory University School of Medicine, Atlanta, GA; Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL; Atlanta Cancer Care, Atlanta, GA; University of Texas Health Science Center San Antonio, San Antonio and University of Wisconsin.

Body: Background: Increased signaling through growth factor pathways including PI3K/Akt/mTOR and HER2 have been implicated in hormone resistance. Everolimus (EVE) improves outcomes when added to endocrine therapy for patients with HR-positive MBC. This study evaluated the efficacy of everolimus (EVE) and trastuzumab (TRAS) in hormone refractory HER2-normal metastatic breast cancer.

Methods: Eligible patients had HR-positive, HER2/neu-negative (IHC +1 or +2, HER2-non-amplified) MBC that had progressed within 6 months of the most recent endocrine therapy. Patients continued on the most recent endocrine therapy they received and were randomized to receive EVE 10 mg oral daily or TRAS IV (8 mg/kg loading dose followed by 6 mg/kg every 3 weeks). At progression, the other agent was added (TRAS in the EVE arm and EVE in the TRAS arm). Patients were followed until disease progression or death.

Results: 54 eligible patients were included in the analysis, and were randomized to EVE (n=30) or TRAS (n=24). 33% of patients were on fulvestrant, 31% exemestane, 22% tamoxifen and 7% letrozole, which were continued. The median PFS was 5.7 months for EVE vs. 2 months for TRAS until first progression or death with hazard ratio of 0.45 (95% CI 0.25-0.81, p=0.008). Among 48 patients who had disease progression, EVE was added to 16 patients who were originally treated by TRAS, and TRAS was added to 12 patients who were originally treated by EVE; the median time to the second progression was 6.3 months for the arm where EVE was added vs. 3.1 months in the arm where TRAS was added. Three patients were taken off study due to decrease in ejection fraction.

Conclusions: This trial demonstrates the efficacy of EVE alone or in combination with TRAS in patients with hormone refractory HR-positive, HER2-negative metastatic breast cancer, who remained on the endocrine therapy they had experienced disease progression on. This suggests that mTOR inhibition has the potential of restoring sensitivity to endocrine therapy and potentially allows the re-use of endocrine agents. Updated results and correlative studies will be presented. Clinical trial information: NCT00912340.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-16-04

Title: Real World data and patterns of care of metastatic breast cancer (MBC) in Brazil: First results of LACOG 0312 retrospective study

Barrios CH H, Uema D, Cronenberger E, Lima V, Bines J, de Sant'ana RO O, Batista ML L, Dybal V, Liedke P, Beato C, Nerón YV V, Giacomazzi J, dos Santos L, Ismael G, Azambuja A, Andrade D, Rosa DD D, Borges G, Mano M, Martinez-Mesa J, Zaffaroni F and Werutsky G. PUCRS Scholl of Medicine - Centro de Pesquisa em Oncologia, Porto Alegre, RS, Brazil; Instituto do Câncer do Estado de São Paulo (ICESP), São Paulo, SP, Brazil; Centro Regional Integrado de Oncologia (CRIO), Fortaleza, CE, Brazil; A.C. Camargo Cancer Center, São Paulo, SP, Brazil; Instituto Nacional do Câncer (INCA), Rio de Janeiro, RJ, Brazil; Hospital Haroldo Juaçaba - Instituto do Câncer do Ceará, Fortaleza, CE, Brazil; Núcleo de Oncologia da Bahia (NOB), Salvador, BA, Brazil; Clínica AMO - Assistência Multidisciplinar em Oncologia, Salvador, BA, Brazil; Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, RS, Brazil; Hospital Amaral Carvalho, Jaú, SP, Brazil; Centro de Pesquisa Clínica – CEPON, Florianópolis, SC, Brazil; Hospital Tacchini, Bento Gonçalves, RS, Brazil; Instituto de Ensino e Pesquisa São Lucas, São Paulo, SP, Brazil; Centro de Hematologia e Oncologia de Rio Claro, Rio Claro, SP, Brazil; Instituto do Câncer Hospital Mãe de Deus, Porto Alegre, RS, Brazil; Instituto Oncológico de Ribeirão Preto (InORP), Ribeirão Preto, SP, Brazil; Hospital Moinhos de Vento, Porto Alegre, RS, Brazil; Centros de Novos Tratamentos Itajaí, Itajaí, SC, Brazil; Instituto do Câncer do Estado de São Paulo (ICESP), São Paulo, SP, Brazil; Faculdade Meridional IMED, Passo Fundo, RS, Brazil and Latin American Cooperative Oncology Group (LACOG), Porto Alegre, RS, Brazil.

Body: Background

Randomised clinical trials (RCT) are considered a gold standard generating efficacy and safety data supporting drug approval. However, real world data (RWD) reflecting health care delivery is becoming increasingly important. RWD on patient profiles and patterns of care in MBC are scarce in developing countries. As an example, observational studies suggest that despite guideline recommendations clearly indicating ET for hormone receptor positive MBC, a considerable proportion of patients in clinical practice begin chemotherapy in early lines of therapy. This pragmatic information addresses the uptake and applicability of the RCT results and should be able to help informing health care planning complementing RCT generated data. The objective of this study is to describe patient characteristics and evaluate actual physician-reported treatments for MBC in Brazil.

Methods

This analysis addresses the first 362 patients included in LACOG-0312, a retrospective study planning to recruit over 700 patients (cut-off date April 30th 2016) with recurrent locally advanced or MBC diagnosed in 2012 in 18 institutions across Brazil. Patient characteristics, type of health insurance coverage, treatment and survival outcome were analysed.

Results

Median age at BC diagnosis was 53 years and 37% were premenopausal. Regarding the educational level, 63.2% had completed elementary (primary) schooling, 75.7% were covered by the public health system while 24.3% had some form of private coverage. 70% of patients had hormone receptor positive (HR+) and 18% had HER2 positive tumors. Median disease free survival time from surgery was 29 months. Interestingly, 30% of patients underwent a biopsy of a metastatic site. Of the 362 patients, 349 (96.9%) received some form of palliative systemic therapy. Median time from diagnosis of metastatic disease to first-line therapy initiation was 46 days but a significant difference was noted between patients with public versus private health insurance (50 vs. 33 days $p=0.012$). Half of the patients received at least 3 lines of therapy (chemo or endocrine) to a maximum of 9 lines. In patients with HR+ tumors, endocrine therapy was administered in 47% in first, 65% in second and 61% in third-line, respectively. Median overall survival (OS) from diagnosis of metastatic disease was 34 months (CI 95%: 25.7-44.3) and no differences in OS were observed between patients with public or private coverage (34 months vs. 35 months $p=0.808$). Causes of death were cancer in 85.2% of patients and treatment toxicity in 3.6%.

Conclusion

Our study included a population with predominantly low educational level and mostly public health insurance. This likely corresponds to the majority of cases and reflects cancer care patterns in Brazil and many developing countries. A considerable proportion of patients were premenopausal at MBC diagnosis. More than half of HR+ patients received at least 3 lines of endocrine therapy although 54% of them had chemotherapy as the first systemic treatment. Patients from the public health system experienced a delay in starting first-line therapy but this didn't seem to jeopardize cancer outcomes in this setting.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-16-05

Title: Early utilization pattern of palbociclib 1 year post-approval in the United States

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Body: Background

Palbociclib was approved in the U.S. in February 2015 for the treatment of advanced/metastatic breast cancer (MBC) in combination with letrozole as initial endocrine based therapy for post-menopausal women with ER+/HER2- disease. We examined the demographic, clinical characteristics and treatment patterns of patients initiating palbociclib (PAL) + letrozole (LET) in real-world, community oncology practices.

Methods

This was a retrospective observational study of female breast cancer patients identified in the Navigating Cancer (NC) EMR database. The NC database collects EMR data, in both structured and unstructured fields (patient/clinical progress notes), from over 975 oncology and hematology providers across more than 50 locations in 25 states. Female patients with record of treatment with PAL after 01/31/2015 were selected. Combination treatment with LET was defined as having a record for LET within 30 days of the PAL prescription. Line of therapy (LOT) was assessed from the date of metastatic diagnosis and assigned by evaluating treatment plans pre-and post PAL initiation. Bi-monthly cohorts were constructed based on the month of initiation of PAL from 02/01/2015-01/31/2016. Interim results are presented; data from an additional three months of follow-up (through 03/31/2016) are pending.

Results

Overall, 931 unique patients were identified as having initiated PAL treatment. Of those, 608 (65.3%) received PAL + LET. Mean follow-up was relatively short at 5.4 mo (SD=3.5). Confirmed ER+/HER2- was observed in 71.6% of patients and 50.3% were age ≥ 65 , mean age was 64.3 yrs. Of patients with available ECOG-PS at treatment initiation (n=424): 0/1=78.5%, 2=17.5% and 3=4.0%. Of patients with a known starting dose (n=418), 69.9% initiated with PAL 125mg, 22.0% at 100mg and 8.1% at 75mg. Compared to women < 65, women ≥ 65 were more often started with 100mg (25.4% vs. 18.9%) and 75mg (10.0% vs 6.5%). Any dose reductions were observed in 20.6% of patients (21.5% of patients receiving 125mg). During the year following approval, 39.8% of patients initiated PAL + LET at LOT1, 15.6% at LOT2, 13.0% at LOT3 and 31.6% at LOT4+, following MBC diagnosis. Over time the proportion of late use (LOT4+) declined from 39.7% in Feb/Mar '15 to 23.9% in Dec '15/Jan '16 with more patients utilizing in LOT3 (from 7.9% in Feb/Mar '15 to 19.5% in Dec '15/Jan '16).

Number of Patients Initiating PAL + LET by LOT and Month.

	All	Feb/Mar '15	Apr/May '15	Jun/Jul '15	Aug/Sep '15	Oct/Nov '15	Dec'15/Jan '16
N (%)	608(100)	63(10.4)	108(17.8)	125(20.6)	108(17.8)	91(14.5)	113(18.6)
LOT1 (%)	242(39.8)	23(36.5)	45(41.7)	53(42.4)	45(41.7)	33(36.3)	43(38.1)
LOT2 (%)	95(15.6)	10(15.9)	12(11.1)	19(15.2)	15(13.9)	18(19.8)	21(18.6)
LOT3 (%)	79(13.0)	5(7.9)	10(9.3)	18(14.4)	12(11.1)	12(13.2)	22(19.5)
LOT4+ (%)	192(31.6)	25(39.7)	41(38.0)	35(28.0)	36(33.3)	28(30.8)	27(23.9)

Conclusions

There was a trend toward earlier utilization of PAL + LET from Feb-Jul '15, an increase in later use during Aug/Sep '15, and a return towards earlier use in subsequent cohorts reaching the lowest proportion of LOT 4+ use observed in Dec '15/Jan '16. After a mean follow-up of 5.4 mo, 21.5% of patients receiving the 125 mg dose had a dose reduction. Final results, with additional follow-up, will be presented at conference.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-16-06

Title: Maintenance immunotherapy in estrogen receptor negative (ER-) locally advanced (LA) and/or metastatic breast cancer (MBC). Long-term follow-up of a phase II study

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Body: Background: Cancer immunotherapy is revolutionizing treatment today. While MBC with estrogen receptor positive (ER+) tumors, responding or stabilizing after first line chemotherapy, may have a benefit from a maintenance endocrine therapy combined with the monoclonal antibody bevacizumab, no option exists for ER- patients. In the past, we have shown, that a combination of 13-cis retinoic acid (RA) and interleukin-2 (IL-2) was able to improve the immune function [enhancing lymphocyte and natural killer cell (NK) counts, and CD4⁺/CD8⁺ ratio] of cancer patients who had had a clinical benefit from chemotherapy (Clin Cancer Res 2001;7:1251). With the aim of improving the immune function which eventually would lead to improvement of progression free survival (PFS) and overall survival (OS), patients with ER- LA or MBC, with a clinical benefit from chemotherapy, were treated with a combination of IL-2 and RA. **Methods:** Eligible patients with ER-, LA or MBC, and no evidence of progression after induction chemotherapy received the following immunotherapy: IL-2, (1.8 M UI), and oral RA (0.5 mg/kg), 5 days/week, 3 weeks/month for 1 year. Therapy was continued, with intermittent schedule until progression. Primary endpoints were the differences in lymphocyte , natural killer cell (NK) counts, and CD4⁺/CD8⁺ ratio, secondary end points were progression free survival (PFS), overall survival (OS) and toxicity. **Results:** From 12/1996 to 04/2009, 74 patients with ER-, LA (49%) or MBC (51%) were entered into the study. Median age was 55 years (range 31-75), 31% of patients were pre-menopausal. Each patient had received a median of 11 courses of first line and salvage chemotherapy regimens (total 850 courses). In addition, 31 patients had received high-dose chemotherapy with peripheral blood progenitor cell transplantation. Disease sites were as follows: soft tissue 54%, viscera 28%, bones 18%. After a median follow-up of 118 months (range 84-232), a total of 374 courses of immunotherapy were delivered. No WHO grade 3 or 4 toxicity was observed; grade 2 cutaneous toxicity and autoimmune reactions occurred in 19% and 16% of patients, respectively. A Statistically significant improvement with respect to baseline values was observed in the number of lymphocytes ($p < 0.001$), NKs ($p < 0.001$), and the CD4⁺/CD8⁺ ratio ($p < 0.01$). Median PFS and OS were 44.9 months and 104.8 months, respectively. **Conclusions:** Maintenance immunotherapy with IL-2 and RA in ER-LA or MBC patients who do not progress after induction chemotherapy is well tolerated, improves lymphocyte, NK, CD4⁺/CD8⁺ ratios, and seems to delay disease recurrence.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-16-07

Title: Analysing the factors affecting survival of breast cancer patients with leptomeningeal metastasis

Niwinska A, Rudnicka H, Kunkiel M, Jagiello-Gruszfeld A and Nowecki Z. The Maria Sklodowska-Curie Memorial Cancer Center, Warszawa, Poland.

Body: AIM: To assess those factors which affect survival in three groups of breast cancer patients with leptomeningeal metastasis (LM) treated at the Warsaw Cancer Center in Poland. Subject groups were: 187 patients consecutively referred during 2003-2015 (group A), 69 patients with LM as the first/only site of dissemination of the disease (group B) and 27 patients who survived for more than 1 year (group C).

Methods: Univariate and Cox multivariate analysis were performed using the following variables: age, Karnofsky Performance Status (KPS), biological subtype of breast cancer (ER/PR+HER2- vs. HER2+ vs. ER-PR-HER2-), systemic intravenous/oral treatment, intrathecal treatment, radiation therapy and the number of treatment methods.

Results. Median survival of patients in group A was 17 weeks, in group B 18 weeks ($p=0.72$) and in group C 19.2 months. In group A, factors positively affecting survival were: KPS \Rightarrow ($p=0.01$), older age ($p=0.003$), biological subtype ER/PR+HER2- ($p=0.003$), systemic intravenous/oral treatment ($p=0.0001$), intrathecal treatment ($p=0.001$) and radiation therapy ($p=0.001$). In the group B, the most important factors affecting survival were a KPS \Rightarrow 70 ($p=0.001$), biological subtype ER/PR+HER2- ($p=0.04$), systemic therapy ($p=0.001$) and intrathecal therapy ($p=0.001$). For group C, older patients ($p=0.01$) with ER/PR+HER2- breast cancer ($p=0.05$, HR 0.18) and after systemic intravenous/oral treatment ($p=0.02$) had higher a probability of survival after 1 year.

Conclusions: Those breast cancer LM patients who were older, having high KPS, with luminal breast cancer and treated with systemic intravenous/oral therapy had a higher probability of survival than the rest of the group. Based on the presented data, the role of intrathecal therapy could not be exactly defined.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-16-08

Title: The impact of genomic mutation on metastatic breast cancer treatment: A retrospective clinical trial

Yuan Y, Yost S, Yuan Y-C, Liu Z, Frankel P, Nicola S and Mortimer J. City of Hope.

Body: Background: Next-Generation Sequencing (NGS) has made genomic mutation-driven cancer medicine feasible. Recognizing the importance of pathway and biomarker-driven personalized therapy for patients with metastatic breast cancer (MBC), we frequently submit tumor tissue for FoundationOne® genomic sequencing. Here we report the results and clinical impact of this test in 44 patients with MBC.

Patients and Methods: An institution IRB protocol was established for this retrospective clinical trial performed at the City of Hope Comprehensive Cancer Center from January 2014 to May 2016 with available tumor genomic DNA mutation results through FoundationOne® testing. Patients' clinical characteristics including age, race, treatment history, clinical outcome and genomic mutation profiles were reviewed.

Results: We identified 44 patients with MBC submitted for FoundationOne® genomic profiling: 24 triple negative breast cancer (TNBC), 16 estrogen receptor positive (ER⁺) and 4 human epidermal growth factor receptor 2 positive (HER2⁺). A total of 23 patients received over 3 lines of chemotherapies prior to FoundationOne® testing. Actionable mutations were identified in 42 of the 44 patients and 23 patients (52%) initiated mutation-driven targeted therapies. Of these 23 patients treated, a total of 17 had accessible responses and 6 patients did not have accessible responses due to short exposure (<2 weeks) and transition to hospice. The remaining 19 patients failed to initiate targeted therapy: 7 transitioned to palliative care/hospice, 5 were placed on other chemotherapy by treating physician, 4 had exhausted all of the targeted therapies recommended, and 3 chose not to start on treatment. Of the 7 responders, 2 received pazopanib and 5 received everolimus containing regimen. Durable response was observed in 3 cases: two patients carried PIK3CA alterations and were treated with everolimus, and the other responder had FGFR1 amplification and was treated with pazopanib. Comparing the genomic mutation profiling with The Cancer Genome Atlas (TCGA) database which contains primary breast cancer, the heavily pretreated TNBC tumors carried higher percentage of PIK3CA mutations (29% vs. 8%, $p < 0.01$).

Conclusion: Targeted genomic sequencing through FoundationOne® can identify effective therapy that has not generally been used based on pathology type. NGS should be performed early in patients with good performance status. This approach should be utilized in a setting where genomic mutation driven therapeutic trials are available.

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2016 San Antonio Breast Cancer Symposium

Publication Number: P6-17-01

Title: Adjuvant treatment with zoledronic acid (ZOL) in stage II/III breast cancer. The AZURE trial (BIG 01/04) 10 year follow-up

Coleman R, Collinson M, Bell R, Marshall H, Dodwell D, Keane M, Gil M, Gregory W and Cameron D. University of Sheffield, Sheffield, United Kingdom; University of Leeds, Leeds, United Kingdom and AZURE Investigators.

Body: Background: The AZURE trial is an academic study designed to determine whether treatment with ZOL added to standard adjuvant therapy improves DFS and time to bone metastases in a broad range of patients with stage II/III breast cancer. Previous analyses after 752¹ and 966² events showed that, despite a reduction in the risk of developing bone metastases, there was no effect on overall breast cancer recurrence. However, preplanned subset analyses identified benefit in women who were in established menopause at study entry, an observation which has subsequently been confirmed by the EBCTCG meta-analysis of >18,000 women included in randomized trials of adjuvant bisphosphonates with reductions in risk of bone metastases and breast cancer mortality of around one third and one sixth respectively.³ All patients in AZURE are now >10 years since randomization and we will present the long-term effects of zoledronic acid on disease relapse, site of recurrence and overall survival.

Materials and methods: Between September 2003 and February 2006, 3360 patients from 174 centres were randomized to receive (neo) adjuvant chemotherapy (CT) and/or endocrine therapy (ET) +/- ZOL 4mg iv every 3-4 weeks for 6 doses, then 3 monthly x 8 and 6 monthly x 5 to complete 5 years treatment. Thereafter patients were reviewed annually until 10 years. Follow-up investigations were as clinically indicated with no protocol mandated imaging to identify sub-clinical disease. Dates of recurrence were backdated to the first clinical suspicion of relapse rather than the date actually confirmed.

Results: Baseline patient demographics, disease characteristics and treatment type were well balanced. 3207 patients (95%) received (neo) adjuvant CT (93% anthracyclines, 23% taxanes). 152 patients received ET alone. As of the datalock on June 7th 2016, with a maximum follow up of ~120 months, there have been 1137 DFS, and 910 OS events. The effects of ZOL on these outcomes, sites of recurrence as well as interactions between treatment effects and both menopausal status and age at study entry will be presented. Further follow up has not identified any new safety concerns. 30 confirmed cases of osteonecrosis of the jaw (ONJ) in the ZOL arm have occurred. There have been no reports of atypical femoral fracture.

Discussion: AZURE is one of the largest phase III studies of adjuvant bisphosphonates, and the first to report results with 10 years of follow-up.

¹Coleman RE. NEJM 2011; 360(7):679-691; ²Coleman RE et al. Lancet Oncology 2014; 15:997-1006; ³EBCTCG Lancet 2015; 386:1353-1361.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-17-02

Title: Evaluation of the impact of oncoprote DX test results on systemic therapy in breast cancer patients at the BC cancer agency (BCCA)

Cheng V, Markarian A, de Lemos M and Schaff K. BC Cancer Agency, Vancouver, BC, Canada.

Body: Introduction: The BCCA started funding the Oncotype DX genomic test for node negative, hormone receptor positive and HER2 negative early breast cancer patients in April 2014. Individual requests for the test are reviewed and approved based on predefined BCCA specific eligibility criteria which are, any size grade 3 cancer and any grade 2 cancer over 1cm (later amended to any size) in women 80 and younger, and any grade 1 cancer in women 40 and younger. General consensus is the test result, or Recurrence Score (RS), can be used to guide whether patients should receive endocrine therapy alone (low RS[LRS] <18) or chemoendocrine therapy (high RS[HRS] >30). For patients with intermediate RS (IRS), there is no consensus on whether the benefits of chemotherapy outweigh the risks of potential toxicities.

Objectives: To determine (1) the concordance of RS with treatments given to patients with LRS or HRS, (2) the treatments given to patients with IRS, and (3) the reasons for requesting the genomic test for patients outside the eligibility criteria.

Methods: This was a retrospective, multi-centre analysis of breast cancer patients for whom the genomic test was requested between June 1, 2014 and May 31, 2015. Charts were reviewed to determine the RS and the therapy given following knowledge of the RS. The primary outcome was the concordance rate between LRS and receipt of endocrine therapy only, and HRS and receipt of chemoendocrine therapy. The discordance rate between RS-based recommendations and treatments given, and the type of therapy received by patients with IRS were also explored. Treatments for those with low IRS (18-24) or high IRS (25-30) were examined separately. Finally, we examined the reasons for requesting the genomic test outside eligibility criteria.

Results: 435 requests were received during the study period. 395 requests were approved and test results were not found in 20 cases. Among the 375 RS results, 191 were LRS (51%), 122 were IRS (33%), and 62 were HRS (16%). The concordance rate between RS low and high and given treatments was 96%. Reasons for discordance included: patients with HRS refusing chemotherapy (n=3), patients with LRS receiving chemotherapy (n=3), patients refusing systemic treatment (n=3), and no explanation (n=1). Among the 122 patients with IRS, 81 were low-IRS and 41 were high-IRS. Overall, 27 % of patients with IRS received chemotherapy. Chemotherapy was given in 16 patients (19.7%) with low-IRS and 17 patients (41.4%) with high-IRS. The main reasons for requesting the test for patients outside eligibility criteria were: grade 1 tumors in patients older than 40 years old (n=20), node-positive disease (n=9), and grade 2 tumors less than 11 mm (n=3).

Conclusion: Our results showed that the majority of patients with LRS or HRS were treated in accordance to RS-based recommendations. When treatment differed from RS-based consensus, the main reason was treatment refusal by patients. Only a quarter of patients with IRS received chemotherapy. Ongoing prospective trials will determine the utility of IRS in defining optimal therapy. Patients denied for the test were older and with grade 1 tumors, or with node-positive disease.

2016 San Antonio Breast Cancer Symposium

Publication Number: P6-17-03

Title: The importance of the ultimate ratio of Omega-6 to Omega-3 fatty acids in the efficacy of fish oil supplements in suppressing inflammation in obese postmenopausal women

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Body: Over the last decade, a large body of evidence has established that obesity is associated with a worse breast cancer prognosis for both pre- and postmenopausal women. There are several mechanisms which have been proposed for promoting this effect, including stage of diagnosis and co-morbidities, but more recent evidence suggests that the obese state is associated with changes in the biology of the disease, promoting a more aggressive phenotype. Our recently published *in vitro* and retrospective studies suggest that this is due, at least in part, through cyclooxygenase 2 (COX-2)-derived prostaglandin E2 (PGE2), and that interventions that suppress COX-2 PGE2 production may provide significant benefit for the obese ER+ patient in preventing many of the cancer-promoting effects associated with obesity. Omega-3 fatty acids have demonstrated anti-cancer benefit through multiple mechanisms, including suppression of inflammation-related signaling. DHA and EPA (omega-3 PUFAs found in fish oil) modulate inflammatory responses through COX-2 dependent and independent mechanisms. However, previous studies investigating the potential anti-cancer benefit of omega-3 PUFA and fish oil supplementation have produced mixed results, and none have focused specifically on the obese patient population.

To determine if supplementation with non-steroidal anti-inflammatory drugs (NSAIDs), including omega-3 fatty acids, can effectively suppress PGE2 production in the obese postmenopausal patient, we conducted a double-blind, prospective Phase 0, comparative, 30 day, non-interventional study with correlative biomarker endpoints. One hundred twenty (120) postmenopausal women without breast cancer were randomized to three arms 1. ASA 81mg po daily, 2. 1500mg of docosahexaonic acid (DHA) and 2500mg eicosapentanoic acid (EPA) given daily and 3. Combined ASA and DHA/EPA at above doses. Serum samples were collected prior to and on day 29 of taking the supplements. PGE2 levels in the pre- and post-supplement serum samples were analyzed in triplicate by ELISA and presented as the percentage of change between post- and pre-supplement levels. Of the women in Arm 2 (DHA + EPA only), only 55% demonstrated a significant suppression of PGE2 levels after 30 day of supplements, compared to those in Arm 1 (ASA), in which 80% demonstrated a significant response.

We anticipate that the omega-3 fatty acid supplements were not as effective in as large a population as the aspirin due to a failure to reach a critical ratio between circulating levels omega-6 and omega-3 fatty acids, which has been shown by our own group and others to be a key determinant of cellular response. Studies are on-going to analyze the PUFA levels in both the pre and post supplement serum samples, and pre-clinical studies are being conducted to determine if the ratio of omega-6 to omega-3 PUFAs modulates PGE2 production in several different cell types, including macrophages, adipocytes and the breast cell itself. These results will be critical for moving clinical studies utilizing these agents forward, both in terms of elucidating the mechanism mediating an effect, and also in identifying an accurate biomarker for monitoring compliance and response.

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Title: Mechanisms of drug sensitization by DHA on doxorubicin-resistant human breast adenocarcinoma

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Body: The major reason for therapeutic failure of chemotherapeutic drugs is the development of resistance against anticancer agents used. Marine derived docosahexaenoic acid (DHA), a non toxic natural lipid (approved by FDA as generally recognized as safe (GRAS) category), has been shown to enhance the cytotoxicity of various anticancer drugs to a variety of cancer cell lines or tumors in animal models. Chemotherapeutic drugs in combination with DHA has also shown better outcomes in clinical trials. We have recently demonstrated that DHA enhanced the chemosensitization of doxorubicin-resistant human breast adenocarcinoma MCF-7 cells (MCF-7-Dox) to doxorubicin. However, the mechanisms by which DHA exerts its chemosensitizing effect remains nascent. This promoted us to explore the possible mechanisms of action of DHA in chemosensitization of MCF-7-Dox cell line. MCF-7-Dox cells were treated either with doxorubicin and DHA alone or in combination. Doxorubicin in the presence of DHA enhanced MCF-7-Dox cell death via inhibition of cell proliferation and invasion, accumulation of doxorubicin within the cells, cell cycle arrest at G2/M phase, and by inducing apoptosis. Combined treatment also reduced the levels of P-glycoprotein (P-gp)- a classical multi drug resistance (MDR) transporter, and TG2- a tumor survival factor under stress condition, and increased levels of lipid peroxidation and pro-apoptotic markers Bak1 and caspase 3. These findings are of potential clinical importance, as DHA might be a promising natural adjuvant drug in treating chemotherapy-refractory breast cancer.

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Title: Site-specific activation of curcuminoids in the breast cancer bone metastases microenvironment

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Body: The majority of women with advanced breast cancer (BCa) develop incurable osteolytic bone metastases. Our laboratory has previously demonstrated that curcuminoids, bioactive compounds isolated from turmeric rhizomes, prevent the development of lytic bone lesions in a murine xenograft model of human BCa bone metastasis and inhibit tumor cell secretion of TGF β -stimulated parathyroid-related protein (PTHrP), a signaling pathway known to drive bone metastasis progression. In both humans and mice, glucuronidated phase II metabolites of curcumin, which are thought to be biologically inactive, are the primary form detected in circulation after consuming curcuminoid-containing foods or supplements. This has led to an untested postulate that curcuminoids may be deglucuronidated at sites of action to form bioactive, aglycone (free) curcumin. Studies were therefore undertaken to test this hypothesis in the context of the murine BCa bone metastasis model, assessing site-specific deconjugation of curcuminoids in the tumor-bone microenvironment.

Effects of curcumin-glucuronide (G-CURC) vs. curcumin (CURC) on TGF β -stimulated PTHrP secretion by bone-tropic MDA-MB-231 (MDA) cells was determined by immunoradiometric assay. G-CURC and CURC levels were quantified by LC-MS in plasma and bone marrow specimens isolated from curcuminoid-treated female nude mice. Endogenous β -glucuronidase enzyme expression was localized by immunohistochemical (IHC) staining in paraffin-embedded sections of decalcified, MDA metastases-containing hind limbs of nude mice inoculated, via intracardiac injection 21 days prior, with MDA cells. Glucuronide deconjugation activity of bone marrow lysates was determined by colorimetric assay.

In contrast to the inhibitory effects of a naturally occurring curcuminoid mixture or pure CURC, G-CURC did not alter TGF β -stimulated PTHrP secretion, confirming its postulated lack of biologic activity. In mice treated with curcuminoids, the majority of circulating curcumin was conjugated (91%). In contrast, the majority of curcumin in the bone marrow (56%) was unconjugated free (aglycone) curcumin ($p < 0.01$). IHC staining of MDA tumor-bearing hind limbs of nude mice demonstrated expression of β -glucuronidase (GUSB), an enzyme that deconjugates compounds in mice and humans, by bone marrow cells, but not by tumor cells. Consistent with IHC, bone marrow from female nude mice displayed significantly higher (30-fold) deconjugation enzyme activity compared to MDA cells ($p < 0.01$). Female C3H/HeJ mice, which possess a partial loss-of-function mutation in the GUSB gene, had significantly decreased (66% lower) deconjugation enzyme activity compared to normal female C57BL/6 ($p < 0.001$) or nude ($p < 0.01$) mice. In addition, treatment with the β -glucuronidase inhibitor, saccharolactone (SL), also decreased deconjugation enzyme activity in marrow lysates from female nude mice.

These results suggest that curcuminoids, in the setting of breast cancer bone metastases, may act as a pro-drug, becoming activated within the tumor-bone microenvironment by glucuronidase-expressing hematopoietic bone marrow cells, to limit the progression of osteolytic lesion formation in a murine model of BCa bone metastasis.

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